ANALYSIS OF PROGRAM ACTIVITIES
NATIONAL INSTITUTES OF HEALTH
1954

NATIONAL CANCER INSTITUTE
VOLUME II

NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

U.S. National Cancer Institute.
Report of program activities.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. Office of the Chief — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. ZC1-400 — SERIAL NO

6. Biological Studies on the Factors Involved in the Development and Growth of Tumors in Experimental Animals. — PROJECT TITLE

7. H. B. Andervont — PRINCIPAL INVESTIGATOR(S)

8. _____ — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

This report deals with progress in studies first reported in the two preceding reports. It is divided into 5 sections, each of which includes a type of tumor arising spontaneously or induced in laboratory mice. These tumors were selected for study because they presented opportunities to study biological factors involved in the development and growth of tumors.

Skin Tumors

This work was started following requests from various investigators who were interested in testing fractions obtained from tobacco for carcinogenic activity. All wished to employ skin painting procedures but there was a lack of evidence concerning the relative susceptibilities of inbred strains of mice to induced skin tumors.

The investigation consisted of the application of a benzene solution of a carcinogenic hydrocarbon (methylcholanthrene) to the skins of the inbred strains maintained in this laboratory. Major findings were described in the report for 1952. Strain I mice were found to be highly susceptible to induced papillomas.

During this year histological diagnoses of approximately half of the induced tumors were obtained slowly. These showed that approximately 90% of the tumors were malignant neoplasms.

Since the primary objective of the experiment was attained this work will be completed when all tumors have been diagnosed. An effort may be made to ascertain the dose response of strain I mice to from 1 to 8 skin paintings of the carcinogen.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

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Liver Tumors

This work was discussed in the last report. A single mouse strain (strain C3H) was used to ascertain the genetic and environmental factors influencing the occurrence of spontaneous and induced hepatomas. It is believed the results of this investigation offer an opportunity to study mechanisms involved in the occurrence of hepatomas in experimental animals.

During the year histologic diagnoses were obtained of a number of these hepatomas which were transplanted during the course of the study. Of 46 hepatomas which were transplanted into C3H mice 42 were carried through 4 to 6 transplant generations. Seven of these hepatomas were transformed to tumors of a different type or revealed interesting growth characteristics. These 7 tumors are discussed in detail.

Six arose in mice that had received carbon tetrachloride. All these primary tumors showed the usual gross and histologic morphology of hepatomas in mice.

The first was transformed to a fibrosarcoma.

The second was divided into 2 lines.

Line A was transformed to a tumor containing areas of fusiform cells with collagenous fibers and also areas of anaplastic tissue.

Line B developed areas showing a glandular pattern with rows of cuboidal cells having no resemblance to hepaticacells.

The third was transformed to a tumor containing both fusiform cells and cells resembling those of the primary hepatoma.

The fourth was divided into 2 lines.

Line A remained a pure heptaoma.

Line B was transformed to a fibrosarcoma.

The fifth developed into a rapidly growing hepatoma which attained a large size within 2 to 3 weeks. It retained the gross and histologic appearance of a hepatoma. It has been sent to various laboratories.

The sixth also developed into a rapidly growing tumor. It changed remarkably in its gross appearance but retained the histologic appearance of a hepatoma.

The seventh tumor arose in a mouse that had received o-aminoazotoluene. The primary tumor resembled the usual hepatoma. Three lines were established. The first remained pure hepatoma. The second became a mixture of hepatoma tissue and a glandular type of tissue. The third became very complex. It contained persisting hepatoma tissue, masses of osteoid tissue and, on occasion, areas of infiltration with normal appearing lymphocytes.

The transformation of hepatomas to other tumor types presents an opportunity to study the biochemical changes involved when the transplanted tumors remain hepatomas or change to a new type of tumor.

Breast Tumors

The preceding report contained the results of an effort to produce breast tumors in mice free of the mammary tumor agent. The experiment is now completed. Male mice free of the mammary tumor agent were castrated at 2 months of age and then given 20 per cent stilbestrol pellets. A variety of inbred strains and their hybrids were tested and of these, mice of strain C3H and F_1 hybrids derived from strain C3H and strains C or R3 were the most susceptible.

It is of interest that the strains and hybrids susceptible to the estrogen induced tumors are also susceptible to tumors produced with the agent. This suggests that the hormones and agent react similarly with the cell before it becomes malignant and explains why, in mice, a combination of hormonal stimulation and agent is most effective in producing tumors. This intricate relationship does not exist between susceptibility to chemically induced tumors. An inbred strain of mice may be resistant to the development of spontaneous breast tumors but susceptible to chemically induced breast tumors and vice versa.

The course of this study is obvious. Now that the appropriate test animals are known efforts to control the development and growth of these hormone dependent tumors may be attempted. These tumors may resemble more closely those occurring in humans. Virtually all spontaneous breast tumors in laboratory mice contain the mammary tumor agent.

Interesting data concerning inbred strain R3 are now available. A litter of these mice was procured in 1945. They were difficult to maintain, developed breast tumors sporadically and were not 100% susceptible to their own transplanted breast tumors. Twenty generations of selective inbreeding were carried out and a vigorous substrain has been developed which shows a breast tumor incidence of virtually 100% in breeding and virgin females. Thus far, I have not found any data concerning the incidence of breast tumors in virgin R3 females. One author states that they develop few tumors but there are no supporting data. It appears that our substrain of R3 mice is as susceptible to the development of breast tumors as is our substrain of C3H.

An agent free line of R3 mice is not developing breast tumors and has proved to be highly susceptible to the agent from wild house mice.

NCI-400

Gastric Lesions

It is regretted further progress on last year's report cannot be given. All mice have been killed and the material is awaiting histologic study. As stated in the previous report lesions of interest were obtained in backcross mice procured by mating strain I to F_1 hybrids obtained from strain I and strain BrS.

The results of our work become more interesting in view of a report from South Africa that a species of mouse has been discovered which reveals a high incidence of gastric cancer.

Testicular Tumors

This work was mentioned briefly in the last report. The objective is to study the development and growth of hormone dependent tumors in an organ which is accessible and offers advantages over other similar tumors in experimental animals.

Strain C males when 2 months of age received a 20% stilbestrol-cholesterol pellet subcutaneously. It was planned to remove the pellets after they had been implanted for 2,4,6,8 or 10 months to ascertain the length of time necessary for the induction of testicular tumors and to determine whether testerone pellets could reverse the changes initiated by stilbestrol.

This procedure could not be followed because many mice developed testicular tumors within 4 to 6 months after receiving the stilbestrol pellets. Consequently the original experimental design was altered and interesting results are being obtained. These are summarized briefly below.

It must be kept in mind that the word "tumor" is used here in its broadest sense and, thus far, none of the tumors has been diagnosed microscopically.

1. The procedure produces a high incidence of testicular tumors. A number of mice develop bilateral tumors. These are excellent test animals for one or both testicles are removed and used for transplantation. Operative procedures with these tumors are extremely easy.

2. The presence of a tumor is easily detected and its growth easy to follow by measuring the size of the testicle.

3. When pellets are removed from mice with testicles 10 mm in length the tumor usually regresses. However, if these mice are given another pellet 4 months after the testicle has receded to normal size they develop a tumor within the same testicle within one month. This suggests that the change toward malignancy has been held in abeyance only by removal of the first pellet and is reactivated by the second.

4. At present it appears that a tumor has reached a critical size when the testicle is 12 mm in length. When pellets are removed from these mice some of the tumors continue to develop and others recede. This indicates that the attainment of independence in these tumors can be ascertained without difficulty.

5. Thus far, tumors have appeared in 22 mice after their pellets were removed. The time between removal of the pellet and appearance of a tumor has been from 1 to 12 months. These mice are of exceptional interest for they show that continuous administration of the estrogen is not essential for the induction of all testicular tumors. Some of these tumors have occurred in mice carrying testosterone pellets.

6. Sixty-nine tumors have been transplanted to determine whether they will grow only in estrogenized hosts (dependent) or whether they are no longer dependent upon the hormone. Of 20 transplanted from mice that were carrying a pellet, 9 failed to grow within a period of 6 months, 11 were dependent and none was independent. These findings are in accord with virtually all reports dealing with dependent tumors in experimental animals.

Of 49 that had continued to grow after removal of the pellets 12 have failed to grow, 18 were dependent and 19 were independent. These findings are not final for many tumors have not been transplanted into first generation hosts for 6 months - an arbitrary time limit for this part of the study.

It is planned to carry each dependent tumor through 4 transplant generations only to see whether it will become independent. When a tumor has proved to be independent it is discontinued. This procedure is essential because of the work and time involved.

The course of this investigation will not be determined until the final results of the preliminary work are known and analyzed.

Up to the present time only three new experiments have been started. The first includes the administration of stilbestrol pellets to 2-month old F_1 males derived by mating strain C animals to other strains maintained in this laboratory. This experiment was undertaken because the strain C mice when given stilbestrol pellets also develop leukemia which is usually confined to the thymus gland. This bothersome, but also interesting reaction, may not be so prevalent in F_1 hybrids.

The second experiment consists of giving stilbestrol pellets to other inbred strain males to ascertain their degrees of susceptibility to testicular tumors and leukemia.

The third experiment is designed to study the influence of age upon the reaction to the pellets. Mice from 1-day to 15-months of age are receiving pellets.

PROJECT REPORT FORM (Cont'd)

10. ACI-450
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	[illegible]	1	5	6	1	5	6	
FY 1956	[illegible]	1	5	6	1	5	6	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Laboratory of Pathology -N.C.I.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM (Cont'd)

15. 201-400
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

None

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

Elected Vice-President of the American Association for CancerResearch.

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Laboratory of Biology
LABORATORY OR BRANCH

3. Cellular Biology Section
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. ZC1- 44
SERIAL NO

6. The Sedimentation Behaviour of Enzymes and Other Substances in Crude Solutions
PROJECT TITLE

7. G. H. Hogeboom and E. L. Kuff
PRINCIPAL INVESTIGATOR(S)

8. M. J. Striebich; A. Meister and L. Levintow (see report of Meister and Levintow)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: This project is an extension of the work described in a preliminary report for the last fiscal year (Project Report No. 10). Its objective is to provide a method whereby the sedimentation behavious of a protein or a complex macromolecular particle may be studied on the basis of a specific chemical property, even though that protein or particle is present in a dilute and crude preparation. In assessing the value of this project, it may be noted that until now the only standardized procedure for the study of sedimentation behaviour has involved the use of the optical ultracentrifuge, which utilizes a non-specific method of analysis (refractive index gradient) and requires relatively pure preparations at high concentration. These disadvantages inherent in the use of the optical centrifuge have been overcome by the use of a new and relatively simple technique of analytical centrifugation.

Methods Employed: Centrifugation is carried out in the horizontal swinging-bucket (SW-39) rotor of the Model E Spinco ultracentrifuge at rotational speeds ranging from 3,000 to 40,000 r.p.m. In order to avoid convective disturbances within the fluid column, it is necessary to accelerate and decelerate the centrifuge at a very slow rate and to use a slight sucrose concentration gradient from the top to the bottom of the fluid column (overall range, 1 to 4 per cent sucrose). With the aid of Mr. Crisp and his co-workers, an instrument was designed and constructed for the reproducible production of uniform and linear concentration gradients, permitting the density and viscosity at any point in the fluid column to be known with accuracy. After centrifugation, the plastic centrifuge tube is carefully removed from the rotor and placed in a hole drilled in

a block of lucite. The tube is then pierced near its base with a hypodermic needle, and a dense solution of sucrose is injected at a slow and constant rate. By this means, the entire fluid column is forced upward and through the perforated base of a plastic cylinder fitted over the top of the tube. Samples are removed, usually at about 2 mm intervals and analyzed for their enzyme or protein content. Sedimentation diagrams are constructed from the analytical data, and the state of dispersity and sedimentation constant of the material under study directly determined.

In a second and somewhat simpler method, the material is centrifuged in the same way, after which the plastic tube is sectioned near its mid-point by means of a pointed knife edge. The concentration of material remaining above the plane of section is determined analytically. This parameter can be related to the sedimentation constant by means of an equation that has been thoroughly tested experimentally. It may be noted that the tube-sectioning technique gives an average sedimentation constant, is thus suitable only for monodisperse compounds, and does not provide the information obtainable from the sampling technique.

Major Findings:

As indicated in last year's report, the study of an extensive series of essentially monodisperse compounds was begun in order to test experimentally the validity of the above technique. This study was completed this year (see publication (a) below). The sedimentation behaviour of compounds with sedimentation constants ranging between 2 and 2,000 Svedberg (the latter particles being visible in the light microscope) was determined by three methods, i.e., by means of the optical ultracentrifuge, the sampling technique, and the tube-sectioning method. The data from all three procedures were in agreement within the expected experimental error. Furthermore, it was shown that the sampling and tube-sectioning techniques were suitable for work with solutions that were too dilute for study in the optical centrifuge.

The next step in the investigation was to turn from preparations of known purity to grossly impure mixtures. For the initial sedimentation studies of crude preparations, it was desirable to select enzymes that had been previously characterized in purified states in the optical centrifuge. Yeast alcohol dehydrogenase and jack bean urease fulfilled these requirements (see publication (b)). The preparations studied were simply extracts of baker's yeast and of jack bean meal in which the enzymes were present in concentrations of the order of 0.005 per cent and comprised less than 5 per cent of the total protein. Alcohol dehydrogenase behaved essentially as a monodisperse compound and gave a sedimentation constant in agreement with values reported in the literature for the cyrstalline enzyme. Urease, on the other hand, was not monodisperse. It was demonstrated, in fact, that four discrete components with urease activity were present and that only 50 per cent of the enzyme sedimented at a rate ascribed to crystalline

urease. As far as we are aware, these experiments comprise the first example of polydispersity of an enzyme that can be highly purified. The method thus provides a direct means of attack on the important question concerning the physical state of enzymes under what might be considered "native" conditions (i.e., before subjection to the rigors of purification).

In collaboration with Drs. Meister and Levintow, a study was made of a partially purified preparation exhibiting glutamine synthetase and glutamyl transferase activities. The main question to be answered was whether the two enzyme activities were associated with the same molecule. By means of tube-sectioning and sampling experiments, it was found that the two activities sedimented at the same rate and behaved as a monodisperse compound. Examination of the preparation in the optical centrifuge revealed that it was a polydisperse mixture of proteins but did contain a component which had exactly the same sedimentation constant as that of the enzyme activities and accounted for 18 per cent of the total protein. It was thus possible to correlate sedimentation data obtained in the optical centrifuge with enzyme activity. It should, of course, be mentioned that two enzyme activities could be associated with different molecules of the same molecular weight and dimensions; this would constitute an unusual situation, however, particularly in view of their high molecular weight ($s_{20,w} = 13.9 \times 10^{-13}$).

Significance to Cancer Research:

An obvious extension of the present technique of analytical centrifugation is the more complete resolution of intracellular structures of various sizes, leading to a study of the distribution of enzymes and other compounds of biological importance among these structural elements of the cell. It has been known for some time, for example, that the "microsomal" fraction is a gross mixture, and there is now some question as to whether the mitochondrial fraction is biochemically homogeneous. It is still our belief that one of the few rational approaches to the cancer problem, as far as the individual malignant cell is concerned, will arise from a complete understanding of the biochemical function of intracellular structures. In another progress report, we have described the initial experiments in the fractionation, by means of the sampling technique, of intracellular particles.

Proposed Course of the Project:

During the next fiscal year, a study will be made of the sedimentation behaviour of a number of apparently soluble enzymes that have not been previously purified or extensively characterized. The two major sources of these enzymes will be isolated mitochondria, and, if time permits, the soluble fraction of the liver cell.

PROJECT REPORT FORM (Cont'd)

10. ZC1- 401
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 14,188	1 1/3	1/3	1 2/3	1 1/3	1/3	1 2/3	
FY 1956	$ 11,048	1	1/3	1 1/3	1	1/3	1 1/3	

12. BUDGET ACTIVITY:

RESEARCH ☑ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

NCI Laboratory of Biochemistry (see work with A. Meister and L. Levintow)

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM (Cont'd)

. NRI-401
SERIAL NO.

. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

(a) G. H. Hogeboom and E. L. Kuff: Sedimentation Behaviour of Proteins and Other Materials in a Horizontal Preparative Centrifuge. J. Biol. Chem., 210: 733-751 (1954).

(b) E. L. Kuff, G. H. Hogeboom, and M. J. Striebich: The Sedimentation Behaviour of Alcohol Dehydrogenase and Urease in Crude Solutions. J. Biol. Chem., in press (Jan., 1955 issue, Vol. 212, 439-448 (1955)).

. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Laboratory of Biology
LABORATORY OR BRANCH

3. Cellular Biology Section
SECTION OR SERVICE

4.
LOCATION (IF OTHER THAN BETHESDA)

5. ZC-402
SERIAL NO

6. The Relation between cell chemistry and cell function
PROJECT TITLE

7. Drs. E. L. Kuff and G. H. Hogeboom
PRINCIPAL INVESTIGATOR(S)

8. Dr. W. C. Schneider and Miss M. J. Striebich
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: To determine the distribution of enzymes and other compounds of biochemical importance in the cells of normal and malignant tissues, with special emphasis on the biochemical properties of particulate cell structures. Recent work in this laboratory (see previous Annual Report) and elsewhere has indicated that the cytoplasmic particulates may fall into a variety of distinct classes, more or less homogeneous within themselves from a biochemical point of view, but differing distinctly from one another. Since further elucidation of such a situation would be of importance in extending our knowledge of cell function, attention has been devoted within the past year to the development and application of highly sensitive and precise methods for attacking this problem.

Methods employed: Size distribution studies of enzyme and nucleic acid containing particles present in tissue homogenates are carried out by means of a procedure which combines centrifugation in a horizontal preparative rotor (Spinco SW-39) with subsequent analysis of samples obtained from the tube contents (the technique is described in detail in the Annual Report entitled "Sedimentation behavior of enzymes and other substances in crude solutions"). Sedimentation is effected in the presence of a sucrose density gradient sufficient to prevent convective disturbances during centrifugation and sampling, but small enough to avoid major osmotic effects upon the particulate material. Wide variations in time and speed of centrifugation permit study of particles with diameters ranging from 0.005 to 0.5 micron.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

NCI-402

Major findings: Work has been carried out with homogenates of rat liver in 30% sucrose. Size distribution analyses have been made of the cytoplasmic particulates exhibiting the following enzymatic activities: succinic dehydrogenase, diphosphopyridine nucleotide (DPN)-cytochrome c reductase, acid phosphatase, and uricase. In addition, the distribution of ribose nucleic acid (RNA) has been studied. All of the succinic dehydrogenase activity was confined to a relatively monodisperse group of particles (mitochondria) with a mean diameter of approximately 0.4 micron. About 30% of the DPN-cytochrome c reductase activity was also contained in these particles; the remainder was associated with a group of much smaller particles, rather polydisperse in size, ranging from 0.03 to 0.15 micron in diameter. All of the uricase activity and approximately 70% of the acid phosphatase activity was associated with particles of a fairly narrow size range, centered about 0.2 micron diameter. RNA, while present in particles up to 0.25 micron in diameter, was associated chiefly with the smaller granules.

Significance to cancer research: The spatial arrangement of enzymes and other materials within the cell is now known to be of critical importance for cellular function. It is therefore hoped that extension of our knowledge of the intracellular distribution of biochemically important components in both normal and malignant cells may reveal differences of such a degree as to provide a rational approach to cancer therapy.

Proposed course of project: The investigation of the size distribution of enzymatically active cytoplasmic particulates will be continued in an effort to determine whether these particulates fall into a limited number of discrete, biochemically homogeneous groups. If such is the case, as appears possible on the basis of results to date, the study will be extended to cells in various phases of growth (e.g., regeneration) and to cancer cells.

Attempts are in progress and will be continued, to correlate the results of centrifugal studies with the findings obtained by electron microscopy of thin sections of liver cells (see Dr. Dalton's report for collaborative electron microscopy).

PROJECT REPORT FORM (Cont'd)

0. AC1-402
SERIAL NO.

1. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$14,188	1	1/3	1 1/3	1 1/3	1/3	1 2/3	
FY 1956	$14,140	1 1/3	1/3	1 2/3	1 1/3	1/3	1 2/3	

2. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

3. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM (Cont'd)

5. AC1-402
SERIAL NO.

6. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

The following papers, dealing with this subject or with related problems are published or in press:

(a) E. L. Kuff: The distribution of fumarase activity in mouse liver homogenates. J. Biol. Chem., 207, 361-365 (1954).

(b) G. H. Hogeboom and W. C. Schneider: The Cytoplasm, in Chargaff and Davidson, THE NUCLEIC ACIDS, Academic Press, New York, in press.

(c) G. H. Hogeboom: Isolation of Cell Components, in Colowick and Kaplan, METHODS IN ENZYMOLOGY, Academic Press, New York, in press.

(d) G. H. Hogeboom: The Isolation and Biochemical Properties of Liver Mitochondria (Symposium on Mitochondria of VIIIth International Congress for Cell Biology) Exptl. Cell Research, in press.

7. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

G. H. Hogeboom: Invited to address Federated Societies for Experimental Biology in the open scientific session, April 12, 1955. Title of address: "Recent Studies of the Relation Between Cell Structures and Cell Chemistry".

PROJECT REPORT FORM

National Cancer Institute (1.) Laboratory of Biology (2.)
INSTITUTE — LABORATORY OR BRANCH

Cellular Biology Section (3.) (4.) ZIA CA-403 (5.)
SECTION OR SERVICE — LOCATION (IF OTHER THAN BETHESDA) — SERIAL NO.

Interrelations between nutrition and the incorporation of metabolites into the proteins and nucleic acids of normal and malignant cells cultured in vitro.
PROJECT TITLE

H. Eagle and G. H. Hogeboom
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objective: As a part of Dr. Eagle's long range program on the nutritional requirements of normal and malignant cells cultured in vitro, we have agreed to study the rate of incorporation of labelled amino acids and other compounds into the proteins and nucleic acids isolated from the structural components of the cultured cells. Preliminary experiments with the Strain L mouse fibroblast have demonstrated that the present technique of cell fraction (as designed for the liver cell) is quite satisfactory for work with the cultured tissue. Some work on the incorporation of radioactive phenylalanine into the proteins of the subcellular fractions has already been carried out. For further details, see Dr. Eagle's Progress Report.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

. LCI-403
SERIAL NO.

. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	0	—	—	—	—	—	—	—
FY 1956	$ 3092	~~2/3~~ 1	—	~~2/3~~ 1	2 1/3	—	1/3	0

. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Section on Experimental Therapeutics, Laboratory of Infectious Diseases,

National Microbiological Institute

. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None.

No entries for 15, 16, and 17

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Laboratory of Biology
LABORATORY OR BRANCH

3. Cellular Biology
SECTION OR SERVICE

4. .
LOCATION (IF OTHER THAN BETHESDA)

5. [illegible]
SERIAL NO

6. Oxidative metabolism in various cellular structures of normal and tumor tissues.
PROJECT TITLE

7. Ruth K. Kielley
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objective: The isolation and purification of xanthine oxidase from the soluble protein fraction of liver cells and the study of the properties of the enzyme in normal and tumor tissues.

Methods Employed: The soluble protein fraction of liver cells was obtained by breaking the cells in a homogenizer and centrifuging the homogenate to remove insoluble proteins. Xanthine oxidase was isolated from the soluble protein fraction and purified by employing methods of protein fractionation which gave the highest yield and purity of the enzyme. These methods included differential centrifugation, ammonium sulfate precipitation, heat treatments, tryptic digestion and calcium phosphate gel adsorption.

Major Findings: A method has been developed for the isolation of highly purified xanthine oxidase from liver which has been applied to the isolation of the enzyme from tumor tissues. Studies on the properties of the enzyme indicate that it is a flavoprotein containing molybdenum and iron and that it catalyzes the oxidation of xanthine or hypoxanthine, reduced diphosphopyridine nucleotides and aldehyde under anaerobic conditions with a dye as electron acceptor. It has been found that xanthine oxidase from Mouse Hepatoma 98/15 oxidizes these substrates under similar conditions at nearly the same relative rates as normal liver and liver from hepatoma-bearing animals.

Significance to Cancer Research: The development of a method for the isolation and purification of xanthine oxidase from liver and the study of the properties of the purified enzyme constitute a basis for comparative studies on tumor tissues. The information gained from these studies not only permits quantitative measurements on the amount of enzyme present in tumor tissues with little

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

interference by side reactions, but provides a basis for determining whether other reactions catalyzed by the enzyme in addition to aerobic oxidation of xanthine, occur and to what extent in tumor tissues. If differences are found between normal and tumor tissue, these differences might be more readily understood and interpreted and perhaps even corrected in terms of the basic requirements of the reactions.

Proposed Research: On the basis of information gained from the study of purified liver xanthine oxidase, it is proposed that previous work on the reduction of dinitrophenol be resumed and developed to include investigations on the role of molybdenum and iron and other cofactors still unknown on one-electron transfer by xanthine oxidase of liver and tumor tissues.

PROJECT REPORT FORM (Cont'd)

AC1-404
SERIAL NO.

BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$9,325	1		1	1		1	
FY 1956	$9,275	1		1	1		1	

BUDGET ACTIVITY:

RESEARCH ☆ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Laboratory of Biology
LABORATORY OR BRANCH

3. Cellular Biology Section
SECTION OR SERVICE

4.
LOCATION (IF OTHER THAN BETHESDA)

5. ZIA-HL-05
SERIAL NO

The morphological aspects of cellular structures isolated from broken cell suspensions.
PROJECT TITLE

A. J. Dalton, G. H. Hogeboom and E. L. Kuff
PRINCIPAL INVESTIGATOR(S)

M. J.Striebich
OTHER INVESTIGATORS

PROJECT DESCRIPTION

The objective of this project, which has been initiated within the last few weeks, is to identify morphologically the intracellular structures released after disruption of the cell membrane and isolation by means of differential centrifugation. The plan of attack will be to examine ultrathin sections of isolated cell structures (after appropriate imbedding and sectioning) in the electron microscope and, by a comparison with sections of whole cells, to determine definitively the morphological composition of isolated cell fractions. For further details of preliminary experiments and projected plans, see Dr. Dalton's Yearly Progress Report.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

9. NC1-405
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	0	—	—	—	—	—	—	—
FY 1956	$6,411	~~1/2~~ 1	1/3	~~1 1/3~~ 2	1/2	1/3	~~1 1/3~~ 5/6	0

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None.

No entries for 15, 16, and 17

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Laboratory of Biology (LABORATORY OR BRANCH)

3. Cellular Biology Section (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. ACI-406 (SERIAL NO)

Electron microscopy of normal and malignant cells.
PROJECT TITLE

Albert J.Dalton and Marie D. Felix
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION

Item A. A study of the Golgi substance with the phase contrast and electron microscopes.

Objectives. The primary aims of this project have been first to visualize and study the Golgi substance in living cells and second, to determine the finer structure of the Golgi substance in fixed and sectioned cells.

Methods employed. Living cells of various types are dissected from the animal separated from stroma by tearing, sealed under a cover slip and examined unstained or after supravital staining with the phase contrast microscope. Photomicrographs are taken for later reference. Tissues from various organs are fixed by vascular perfusion or by immersion, dehydrated, embedded in plastic, sectioned with a microtome adapted for ultra-thin sectioning at approximately 0.02 - 0.05 u and examined with the electron microscope. Photomicrographs are taken and prints made at from 7,000 to 150,000 diameters.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Major findings. The Golgi substance was identified in intact, living cells, followed during its extrusion from damaged cells and identified after extrusion into the surrounding medium. This made possible its identification in homogenates and after isolation by centrifugation using a layering technique (Schneider, Dalton and Kuff). This has made chemical analysis possible.

An analysis of the detailed structure of the Golgi substance of cells of the epididymis was made with the electron microscope. It was found to consist of three distinct and characteristic components not found elsewhere in the cell. Following this analysis, a series of widely different cell types was examined to determine whether this structural arrangement could be considered as typical of mammalian cells generally. While varying in detail, the same basic components were found in all cell types examined. Using a new impregnation technique (Item F), it was found that all three components are involved in the development of the Golgi reticulum or network of classical cytology. Thus, as a result of studies carried out in this laboratory over a period of several years, the question as to whether the Golgi substance actually exists has been answered in the affirmative and the uncertainty as to the true form of the Golgi substance has been removed. These were two questions which have occupied the minds and involved the emotions of cytologists since the first description of the network by Golgi in the late 19th century.

This study on the Golgi substance, now nearly complete, was part of a larger objective. This larger objective is to develop a solid baseline of knowledge and understanding of the structure of normal cells. The field of electron microscopy of tissue sections is so new that this base line is still in the process of being laid down. This base line is essential in order to approach the problem of properly evaluating any basic morphologic, chemical or physiological differences between normal and neoplastic cells.

Proposed course of the project: Studies in classical cytology years ago suggested the possibility that the contractile vacuole of protozoa and sponges is the homologue of the Golgi substance of cells of higher animals. Now that the finer structure of the Golgi substance is known, it will be possible to determine whether this homology really exists. In collaboration with Prof. J. Bronte Gatenby, University Zoological Dept., Trinity College, Dublin, a comparison between the Golgi substance of vertebrate cells and the wall of the contractile vacuole of representative protozoa and sponges is being made. Preliminary results suggest that this homology does hold and this in turn gives a hint as to one of the possible functions of the Golgi substance, that of control of water balance in the cell.

Item B. In collaboration with Drs. Hogeboom and Kuff.

Objectives: The purpose of this project is to use the electron microscope as a means of checking the results of modifications of centrifugation techniques introduced to obtain purer samples of various cell fractions. The use of the electron microscope in this

project is essential since many fractions consist of particles of sizes well below the resolving power of the light and phase contrast microscopes.

Methods employed: Cell fractions obtained after centrifugation are fixed, dehydrated, embedded and sectioned, then analysed with the electron microscope to determine purity of sample.

Major findings: This project has just started.

Significance to cancer research: This project has primarily a service function, i.e., to assist Drs. Hogeboom and Kuff in their studies of the chemical analysis of cell components of normal and neoplastic cells.

Proposed course of project: It is expected that this project will continue along present lines until satisfactory centrifugation techniques are developed.

Item C. In collaboration with Dr. Eagle.

Objectives: The objective of this project is to study the detailed morphologic changes which result from the removal of individual amino acids and vitamins from the culture medium.

Methods employed: Dr. Eagle has developed a synthetic medium consisting of specific amino acids and vitamins plus dialysed horse serum. The removal of any one of these amino acids or vitamins results in cessation of growth and eventual degeneration of the culture. Cells from control cultures will be compared with cells from deficient cultures and with cells recovering from deficiency. Cells grown on cover slips will be studied in the living state with phase contrast and cells grown in flasks will be studied with the electron microscope after fixation, collecting and centrifuging, dehydration, embedding and sectioning.

Major findings: Preliminary studies with phase contrast indicate a reduction in number and diameter correlated with a great increase in length of mitochondria of cells deficient in tyrosine. Preliminary studies with the electron microscope indicate a disappearance of the internal membranes of mitochondria and an increase in number and complexity of arrangement of the double membranes of the ergastoplasm or basophilic substance of cells deficient in tyrosine.

Significance to cancer research: The striking changes demonstrated in these preliminary studies give reason to hope that detailed analysis of the effects of deficiencies in this and other amino acids and vitamins will lead us to an understanding of the part played by these building blocks of protoplasm in the formation of the structural components of cells. Such basic knowledge would be of obvious and great importance in any intelligent approach to the problems involved in the study of the growth and inhibition of growth and multiplication of malignant cells.

Proposed course of project: It is proposed to determine whether a particular deficiency affects neoplastic cells differently from normal cells and further, to determine whether the changes observed to date are characteristic of any deficiency or are specific for each amino acid or vitamin deficiency.

Item D. Comparison of cells from tumors grown as subcutaneous transplants with free tumor cells grown inperitoneal fluids.
Objective: The purpose of this study is to determine what if any differences in cell structure result from growth of tumor cells in these two very different environments.

Methods employed: For subcutaneous tumors, the methods are similar to those described under item A and for free cells in peritoneal fluid, the methods are similar to those described under item C.

Major findings: Based on a comparison with earlier findings, very early and preliminary results suggest that a modification in mitochondrial form and internal structure occurs when cells are grown in peritoneal fluid. There is also an indication of a change in the relative preponderance of the three components of the Golgi complex.

Significance to cancer research: This study is another approach to the problem of determining the factors which modify and control tumor cell growth and multiplication.

Proposed course of project: These findings are merely suggestive and are based on very few experiments. It is hoped that they will be confirmed and expanded in a continuance of this project during the coming year.

Item E.

Objectives: On serial intraperitoneal transplants of melanoma S-91 it was found that a new amelanotic cell type appeared, having the characteristics of a lymphoma, rather than a melanoma cell. This cell outgrew the melanoma cell and eventually replaced it. It seemed possible, by repeating this study, to determine whether the new cell type is a mutant of the melavoma cell of whether it appeared as a malginant transformation of a host cell.

Major findings: The appearance of this new cell type has been observed in 3 separate experiments. It does not grow well in the peritoneal fluid but appears to migrate to the spleen and lymph nodes where it multiplies rapidly to produce a picture characteristic of lymphosarcoma. Subcutaneous or intreperitoneal transplants of fragments of lymph nodes or spleen results again in growth in spleen and lymph nodes in the succeeding generation. It is not an infection and is not transplantable as an ultra-filtrate - whole, viable cells being necessary for successful transfer.

Significance to cancer research: Answers to the problems involved in this project would add to our knowledge relating to the concepts of malignant transformation and mutation of neoplastic cells.

Proposed course of project: Studies with the phase contrast microscope have not given an answer as to the origin of this cell. It is hoped that an analysis with the electron microscope and a genetic analysis will provide the answer.

Item F.

Objectives: The objectives of this project were two. The first was to develop a fixative which would give more satisfactory preservation of blood, peritoneal fluid and tissue culture cells than the fixative now in general use. The second was to develop a method of impregnation of the Golgi substance suitable for studies with the electron microscope.

Methods employed: Using tumor cells and normal cells growing in the peritoneal fluid as test objects for the first objective and cells of the epididymis as test objects for the second objective, several approaches to the problems were made. The appearance of the cytoplasm, nucleus and the Golgi substance on examination with electron microscope being the final check.

Major findings: (A) The fixative. It was found first that K2Cr2O7 brought to neutrality with KOH served as an excellent buffer in the pH range 5.6 to 7.6. It was also found that a mixture of 1% $K_2Cr_2O_7$ brought to pH 7.2 with KOH, 1% osmic acid and 0.85% Na Cl gave excellent fixation of single cells. There was no evidence of leaching out of cytoplasmic or nuclear components after relatively long periods of fixation and dehydration and relatively little damage from polymerization of the embedding matrix. (B) The impregnation method. Second, it was found that fixation with veronal-acetate buffered osmic acid, followed by a short rinse in distilled water, followed by incubation at 37° C. in 2% osmic acid brought to neutrality with a minimum of buffer gave good preservation of cytoplasmic detail and also selectively impregnated the Golgi substance.

Significance to cancer research: The development of the new fixative and the impregnation method have made feasible and guarantee more satisfactory results in the investigations involved in all 5 of the projects previously presented.

Proposed course of project: This project is completed, except for minor modifications under special conditions.

PROJECT REPORT FORM (Cont'd)

10. NCI-406
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 29,182	2	2	4	2	2	4	
FY 1956	$ 29,186	2	2	4	2	2	4	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

15. ACI-406
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Cytologic and cyt Chemical characteristics of the Golgi substance of epithelial cells Of the epididymis - in situ in homogenates and after isolation. A. .Dalton and Marie D. Felix, Am. J. Anat. 94:171-208, 1954.

Electron microscopic observations on the Golgi substance and the ergastoplasm. VIIIth International Congress for Cell Biology, Leiden, The Netherlands, Sept. 1-8, 1954. To be published as part of a symposium as a supplement to Exp. Cell Research

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

Recipient of Anna Fuller Fellowship consisting of a grant to travel to Europe, visit laboratories on the continent and the British Isles to discuss problems of mutual interest with scientists in various laboratories and to attend the VIIIth International Congress for Cell Biology.

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Laboratory of Biology
LABORATORY OR BRANCH

3. Cellular Biology Section
SECTION OR SERVICE

4. .
LOCATION (IF OTHER THAN BETHESDA)

5. ZC1-CB-7
SERIAL NO

Desoxyribosides in Normal and Tumor Tissues
PROJECT TITLE

Dr. Walter C Schneider
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objective:

To determine whether precursors of desoxyribonucleic acid (DNA) are present in normal and cancer tissues, to ascertain the nature of such precursors, and to determine their metabolic significance especially with respect to cancerous tissues.

Methods Employed:

Desoxyribosides and desoxyribotides in the range 0.1-0.5 microgram are determined microbiologically with the organism L. acidophilus R-26. This organism will grow equally well on any desoxynucleoside or nucleotide and is thus useful for general survey work. Paper chromatographic methods have been devised for the separation of desoxyribonucleosides and, coupled with the microbiological assay, are used for the determination of the desoxynucleoside composition of tissue extracts. For more certain identification of nucleosides, the compounds (detected microbiologically) are isolated from several chromatograms, rechromatographed, reisolated, and identified by means of ultraviolet spectra, R_f, and chemical tests for desoxyribose.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Major findings:

Desoxyribosidic compounds have been found to be present in acid extracts of all mouse and rat tissues tested. Normal mouse tissues and blood have very low levels (ca. 1 microgram desoxyriboside per g). Mouse tumors contain from 3 - 30 times as much desoxyriboside. Normal rat tissues and blood contain amounts of desoxyribosides in the mouse tumor range. Rat hepatoma and normal rat liver contain about the same amount of desoxyriboside.

Chromatography of tissue extracts on ion exchangers and on paper and acid hydrolysis of tissue extracts indicated that the compounds present were pyrimidine desoxyribosides. Paper chromatography showed further that the active compounds in tissue extracts corresponded in average R_f to either desoxycytidine, desoxyuridine, or thymidine.

In earlier experiments, compounds behaving like desoxycytidine and desoxyuridine were found to be present in normal rat liver and blood. In a rat hepatoma, however, and in the blood of hepatoma bearing rats, a thymidine like compound was observed in addition. These findings thus indicated a qualitative difference in normal and tumor bearing rats. In some of a large number of recent experiments with rat liver, however, a thymidine like component was observed in the solvent system used in the earlier experiments. The appearance of this component in this solvent system is apparently an artefact. Consequently the earlier experiments with tumors will have to be repeated.

Large scale experiments on isolation of desoxyribosides from rat liver and blood have resulted in the isolation of a compound from blood identical in its properties with desoxycytidine. Colorimetric tests have been obtained for desoxyribose and the amounts of the latter found are equivalent to the amounts of desoxycytidine calculated to be present from the ultraviolet spectrum. A fraction, isolated from rat liver, also shows certain of the spectral properties of desoxycytidine but contains desoxyribose considerably in excess of the cytosine content. This indicates the presence of other desoxyribosides, perhaps desoxyuridine.

An unknown compound has been observed in large scale chromatograms of normal rat blood. It has been detected by its reaction with cysteine-sulfuric acid, a reagent supposedly specific for desoxyribose. Since this compound has no growth promoting activity for L. acidophilus, it would appear to be desoxyribose or another compound with a similar reactive group. It is not present in liver.

NCI-407

Significance to cancer research:

The presence of desoxyribosides in animal tissues is of considerable importance to fundamental biochemistry as well as to cancer research. These compounds may represent the unknown intermediates involved in the synthesis of desoxyribonucleic acid, the generally accepted carrier of the genes. Since the synthesis of DNA must proceed at an abnormal rate in tumors, knowledge of how precursors of DNA are formed and how they are utilized is of considerable importance.

Proposed course of research:

The nature of the desoxyribosides present in tumors, normal mouse tissues, normal rat tissues, and regenerating and embryonic tissues will be investigated. Further studies will also be made on the nature of the unknown compound present in blood. Studies are also contemplated on the metabolism of desoxyribosides in tissues.

PROJECT REPORT FORM (Cont'd)

NCI--407

10. NCI-407
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	CONSIDERED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$ 11,482	1	1	2	2/3	1	1 2/3	
FY 1956	$11,501	1	1	2	2/3	1	1 2/3	

12. BUDGET ACTIVITY:

RESEARCH ☑ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM (Cont'd)

15. 161-407
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

Served as invited chairman of cytochemistry session of American Society of Biological Chemists at Atlantic City, April, 1954.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. Cellular Biology Section — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. ZC1-409 — SERIAL NO

6. Studies on Morphology and Physiology of two lymphocytic tumors. — PROJECT TITLE

7. Emma Shelton — PRINCIPAL INVESTIGATOR(S)

8. _____ — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: To correlate differences in the morphology and physiology of two lymphocytic tumors with the differences in their behavior in the same host.

Methods: The tumors are grown as ascites tumors in the strain A mouse. Freely suspended, living cells may be studied with the phase microscope. The number of cells may be counted in a hemocytometer to give a quantitative significance on a per cell basis to biochemical tests.

Major Findings: The major findings of this project during the past year are of a negative nature. A concerted effort was made to demonstrate the presence of the enzyme hyaluronidase in these two tumors. Although several biochemical assays were employed, it was not possible to demonstrate the presence of this enzyme in these tumors.

Significance to Cancer Research: The two tumors being studied here are unique in that they are vastly different in their ability to invade the hosts tissues despite the fact that they are of similar origin and cell type. It is hoped that a study of these cells from the biochemical and cytological standpoint will yield information as to why some lymphoid cells behave as highly invasive leukemias while others, lacking the ability to disseminate, grow as localized lymphosarcomas.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Proposed course of study:

1. Further studies on the ability of the cells of these tumors to pass through membranes of known pore size.

2. Cinematographic studies on the locomotion *in vitro* of the cells of these two tumors.

10. ACI-408
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 7,295	1/2	1/2	1	1/2	1/2	1	
FY 1956	$ 7,297	1/2	1/2	1	1/2	1/2	1	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

15. 121-408
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:
Shelton, E.: Studies on mouse lymphomas. I. Cytology and nucleic acid content. J. Nat. Cancer Inst. 15: 49-61, 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:
None.

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Laboratory of Biology
LABORATORY OR BRANCH

3. Cellular Biology Section
SECTION OR SERVICE

4. .
LOCATION (IF OTHER THAN BETHESDA)

5. Z01-409
SERIAL NO

Studies on liver carcinogenesis in the mouse
PROJECT TITLE

Emma Shelton
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objectives: The objective of this project is to investigate the changes which take place in the parenchymal cells of the mouse liver prior to the formation of a tumor and to develop quantitative cytological methods which will permit the interpretation of biochemical findings on a "per cell" basis.

Methods employed: The methods employed may be divided into two categories (1) methods designed to induce tumor formation in the mouse liver and (2) methods designed to study the changes in the liver.

Under (1) - Strain A/HeN female mice were fed 0.05% orthoaminoazotoluene in a semisynthetic diet. The effect of the following variations on tumor incidence was studied

a) stilbestrol
b) high riboflavin in the diet
c) use of a cross between A/HeN and A/LN mice

Under (2) Histochemical tests for glycogen, ribonucleic acid, fat, and succinic dehydrogenase were performed on tissue sections of the livers of the mice. Histological stains for reticulum, connective tissue and mitochondria were also employed. In one experiment, mice were killed at 4-week intervals during dye feeding in order to give a more dynamic picture of the alterations which occurred in the liver.

Homogenates of normal mouse and rat liver were used in the development of quantitative cytological methods.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

NCI-409

Major findings: 1. Tumors of the hepatic parenchymal cells can be produced in between 80 and 100 per cent of strain A female mice by adding the stimulus of the female sex hormone, stilbestrol, to the carcinogenic action of the azo dye, orthoaminoazotoluene. When the dye was fed in a semisynthetic diet it was found that:

A. In combination with stilbestrol it was necessary to feed the dye for only 60 days in order to achieve a maximum carcinogenic effect, while without stilbestrol 120 days are required.

B. Tumors begin to appear as early as 28 days after the beginning of dye feeding and in one experiment 100% of the animals had gross liver tumors after 90 days.

C. High riboflavin does not protect the mice against the carcinogenic action of the dye.

D. Genetic constitution of the mouse plays an important role in its susceptibility to the carcinogenic action of the dye.

2. A method was developed for making differential counts of the different types of nuclei in a liver homogenate. This permitted the enumeration of the nuclei of the hepatic parenchymal cells separately from the nuclei of all other cells in the liver.

Significance to Cancer Research: In order to gain a more fundamental knowledge of the cause of cancer more information is needed concerning the biochemical and morphological events which occur in the cell that is undergoing the malignant transformation. This project is composed of a series of studies with a biological system wherein it is possible to investigate the process of carcinogenesis in one type of cell, the hepatic parenchymal cell.

Proposed course of project:

1. To continue to investigate the factors involved in the production of tumors in the mouse liver -- i.e. nutritional, and hormonal as well as effect of dye concentration and the physiological state of the liver.

2. To investigate quantitative changes in the cellular composition of homogenates of liver from azo dye fed mice.

3. To study by histochemical methods some of the changes taking place in the liver during the first few weeks of dye feeding.

PROJECT REPORT FORM (Cont'd)

10. AC/-409
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$7,275	1/2	1/2	1	1/2	1/2	1	
FY 1956	$7,277	1/2	1/2	1	1/2	1/2	1	

12. BUDGET ACTIVITY:

RESEARCH ☑ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Laboratory of Biology
LABORATORY OR BRANCH

3. Cellular Biology Section
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. ZC1-4106(a)
SERIAL NO

Transparent Chamber
PROJECT TITLE

Glenn H. Algire
PRINCIPAL INVESTIGATOR(S)

James M. Weaver
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Developments in transparent chamber techniques.

Objectives:

Methods were described in the previous report, for microscopic observation of cells in diffusion chambers. With these procedures, contrast and resolution of structural detail in cells was impaired by diffusion of light during transillumination through the skin of the mouse. The current objective has been to devise improved diffusion chambers for microscopic study of implanted cells isolated from contact with host tissues by membranes.

Methods:

The transparent chamber is constructed so that light from the microscope condenser passes only through glass or plexiglas before reaching the thin layer of cells within the diffusion chamber.

Major Findings:

These improvements now have made it possible to use either bright-field or phase microscopy, and thus to obtain increased contrast and resolution of structural details of living cells in mice.

Significance to Cancer Research:

These newly developed methods for microscopy applied to "tissue culture

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

in vivo" provide improved means for cell identification and observation of cellular behavior under various experimental conditions, such as in problems of tissue transplantation, carcinogenesis, and in analysis of the mechanism of action of tumor damaging chemicals.

Proposed Course:

These methods will be used in experimental studies of cells in diffusion chambers as described in other projects.

PROJECT REPORT FORM (Cont'd)

0. LCI-4(a)
SERIAL NO.

1. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$4,863	1	2		1/3	1/3	2/3	
FY 1956	$4,865	1	1		1/3	1/3	2/3	

2. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

3. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

15. ACI-410(a)
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Algire, G. H.: Visualizing cellular behavior "in vivo". XVth Congress de la Internationale Societe de Chirurgie, Imprimarie Henri de Smedt, Bruxelles, 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None.

PROJECT REPORT FORM

1. National Cancer Institute INSTITUTE	2. Laboratory of Biology LABORATORY OR BRANCH	
3. Cellular Biology Section SECTION OR SERVICE	4. LOCATION (IF OTHER THAN BETHESDA)	5. ZO1-HP (B2) SERIAL NO

Mechanism of action of chemotherapeutic drugs on tumors.
PROJECT TITLE

Glenn H. Algire
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objectives -

To determine the mechanism of action of various drugs ontumors in terms of their circulatory effects, or direct cytotoxic properties.

Methods Employed -

Microscopic observations in living mice of circulatory reactions to various drugs,.Studies of cellular reactions using diffusion chambers in which implanted cells are isolated from contact with host tissues by porous membranes.

Major Findings -

Subcutaneous injection of podophyllotoxin (as found previously for bacterial polysaccharids) results in profound, prolonged fall in peripheral blood pressure, with decreased circulation through both normal tissues and transplanted sarcomas, but with the most severe reduction of blood flow in tumors. The prolonged ischemia (6-8 hrs) was followed by hemorrhage in the tumors as the blood flow began to improve, and central necrosis of the tumor was the end result. The above phenomena occurred also after mechanical occlusion of blood supply to the tumor area. It was concluded that the acute tumor necrotizing action of podophyllotoxin on solid vascularized tumors was an indirect effect of the decreased blood flow induced by this substance.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Recent preliminary experiments suggest that the effects of this agent on peripheral circulation and in causing tumor necrosis may be counteracted through the use of heat to bring about local increase in blood flow.

Significance to cancer research -

Evaluation of therapeutic agents in cancer depends upon understanding their mode of action. Microscopic observations in living mice, using transparent chamber methods, help to determine whether an agent acts directly upon the living cell or indirectly through its effects on the circulation of the host animal.

Proposed Course of project -

1. To continue the above experiments on control of regional blood flow, in relation to circulatory mechanism of action of tumor necrotizing drugs.

2. Apply the diffusion chamber methods to direct observation of the effects of various chemotherapeutic drugs on cells isolated from host tissues.

PROJECT REPORT FORM (Cont'd)

O. NCI-410(2)
SERIAL NO.

1. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 4,863	1	1	2	1/3	1/3	2/3	
FY 1956	$ 4865	1	1	2	1/3	1/3	2/3	

2. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

3. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

NB1-410(2)
SERIAL NO.

PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Algire, G. H., Legallais, F. Y., and Anderson, B. F.: Vascular reactions of normal and malignant tissues in vivo. VI. The role of hypotension in the action of components of podophyllin on transplanted sarcomas. J. Nat. Cancer Inst. 14: 879-893, 1954.

HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. Cellular Biology Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. Z01-4/0(c) — SERIAL NO

Tumor growth and vascularization. Initial vascularization of transplanted normal and tumor tissues.
PROJECT TITLE

Ruth M. Merwin and Glenn H. Algire
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objectives:

The object of this study is to evaluate the significance of the activity of the graft vessels in normal and neoplastic tissues in the initial vascularization of grafts.

Methods employed:

Microscopic observations were made in living mice using transparent chambers. With the usual method of studying grafts the connections between the graft and host vessels take place underneath the graft. In order to see the establishment of connections grafts were placed so the connections were established beside rather than beneath the grafts.

Major findings:

The vessels in grafts of normal thyroid tissue were active in establishing connections with host vessels. These grafts did not stimulate the host vessels to produce new ones. In grafts of tumor tissues on the other hand, the graft vessels were not active but the grafts stimulated the host vessels to produce many new vessels. The conditions in the connective tissue surrounding the graft greatly affected the growth of new sprouts from both the graft and host vessels.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

NCI-410(c)

Significance:

These findings should aid in placing grafts of tumor and normal tissues and in understanding the causes leading to variations in the time of establishment of grafts. Further the method of placing grafts so the adjacent host vessels can be easily seen is applicable to other problems which would be of significance in understanding the vascularization and establishment of tumor metastases (see proposed course).

Proposed course:

It is planned to compare various types of tumors in their capacity to stimulate host capillary proliferation.

PROJECT REPORT FORM (Cont'd)

10. ~~401-~~ 410(c)
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$4,863	2	1	3	1/3	1/3	2/3	0
FY 1956	$4865	2	1	3	1/3	1/3	2/3	0

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Laboratory of Biology
LABORATORY OR BRANCH

3. Cellular Biology Section
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. ZA CI-411
SERIAL NO

Studies in carcinogenesis using diffusion chamber techniques.
PROJECT TITLE

Glenn H. Algire
PRINCIPAL INVESTIGATOR(S)

James M. Weaver
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objective -

A recently devised diffusion chamber method for "tissue culture in vivo" provides a new approach to certain problems of chemical carcinogenesis. Earle and associates have found that normal adult fibroblasts grown in tissue culture in a completely heterologous media in the absence of deliberately added MCA give rise to sarcomas when inoculated into mice. One possible explanation for these results has been "growth in the heterologous media". This explanation would be ruled out if normal or embryonic mouse tissue underwent a malignant change when enclosed within diffusion chambers.

Goldblatt, following experiments and theories of Warburg (1927), has presented evidence that normal fibroblasts in tissue culture undergo a malignant change if subjected to conditions of intermittent anoxia. Through selecting filters of increasing diameter, and of consequent greater diffusion distance, one could study the effect of this variable on neoplastic change.

The immediate objective is to ascertain whether normal subcutaneous tissue or embryonic tissue enclosed within diffusion chambers, undergoes neoplastic transformation.

Methods Employed -

1. To determine whether the materials (plexiglas II, and Millipore filters) are carcinogenic for mice - (by intraperitoneal and subcutaneous implantation).

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

2. Fragments of muscle from young mice (strain C3H) were enclosed within diffusion chambers and placed intraperitoneally into adult hosts of the same strain. At intervals of from 1 to 3 months these will be reimplanted (open) into new hosts which have received 400-425 r total body irradiation.

Results -

These experiments are now in progress. It has been found that fragments of muscle from young mice produce a vigorous outgrowth of connective tissue cells, with numerous dividing cells. Adult connective tissue cells also migrate out in diffusion chambers.

Proposed course of project -

Future plans: Future plans will depend upon the results of experiments now in progress. In addition to studies on normal tissues, various chemical carcinogens and inactive but related compounds will be used in studies of effects on cells through microscopic observations in vivo in diffusion chambers.

PROJECT REPORT FORM (Cont'd)

9. ACI-411
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$4,863	2	2	4	1/3	1/3	2/3	
FY 1956	$4,865	2	2	4	1/3	1/3	2/3	

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Laboratory of Biology
LABORATORY OR BRANCH

3. General Biology Section
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. ZOI-412
SERIAL NO

Factors in the localization of diffusible dyes in tumors.
PROJECT TITLE

Glenn H.Algire
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objectives -

Moore observed apparent selective localization of fluorescein in brain tumors, 3 to 5 hours after injection of the dye into patients about to undergo surgery. This did not regularly occur in other types of tumors or intumors of sites other than the central nervous system. The mechanism of the localization is unknown, but it was thought to be related to differences in permeability of the capillaries of normal brain tissue as compared to those of neoplastic tissue. The phenomena was said not to be related to the presence or absence of necrosis in the tumor.

The objectives of the following experiments have been to investigate such factors as vascularity, changes in blood supply, cellular reaction, and necrosis in relation to localization of fluorescein in normal and malignant tissues; to enhance the localization of this or other diffusible dyes in transplanted and spontaneous tumors.

Methods Employed -

Microscopic observations in living mice using transparent chamber methods; supplemental gross observation. Fluorescence was elicited by use of CH_4 Mercury vapor lamp with Wood's Filter, for transmission of 3450 A radiation. Observations were made on normal tissues, transplanted and spontaneous tumors. In other experiments drugs were used to alter regional blood flow in tumors and surrounding tissues following injection of fluorescein.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Major Findings -

1. The differential rate of diffusion of dye into and from avascular tissues, as contrasted with richly vascularized tissues, accounts for the apparent selective or preferential localization of dye in certain tumors.

2. Fluorescein was found to localize in transplanted sarcomas approximately 3 hours after injection of the dye along with podophyllotoxin.

3. In transparent chamber preparations these results were observed to be correlated with the decrease in tumor blood supply following podophyllotoxin.

4. Similar results were obtained when subcutaneously transplanted sarcomas (not transparent chamber preparations) were treated in the same way; hence were not related to the "unusual" presence of the transparent chamber; tumors less than one-half centimeter in diameter often failed to show localization.

Significance -

It would appear that localization of fluorescein can be accomplished in tumors and at sites other than of central nervous tissue origin, through the use of drugs which decrease regional blood flow. These observations are of importance in regard to several problems of clinical cancer:

1. Localization of primary or metastatic tumors at operation, through enhancing the accumulation of fluorescein or other freely diffusible dyes, or radioactive tracers intumors of non C.N.S. origin.

2. The possibility, through control of regional blood flow, of enhancing the accumulation in tumors of radioactive chemotherapeutic agents, or chemotherapeutic drugs.

Proposed course of project -

1. Continuation of studies similar to these using transplanted and spontaneous mammary tumors.

2. Application to the problem, of more physiologic methods of modifying regional blood flow.

3. Use of slowly diffusible dyes.

. RCI-412
SERIAL NO.

. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$4,863	1	1	2	1/3	1/3	2/3	
FY 1956	$4,865	1	1	2	1/3	1/3	2/3	

. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Laboratory of Biology (LABORATORY OR BRANCH)

3. Cellular Biology Section (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. ZC1-CB-3 (SERIAL NO)

6. Mechanisms determining susceptibility and resistance to tumor and normal tissue grafts in mice. (PROJECT TITLE)

7. Glenn H.Algire, James M. Weaver (PRINCIPAL INVESTIGATOR(S))

8. Richmond T. Prehn (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Several aspects of immunity to transplanted normal and malignant tissues are covered in this report.

(a) Vascular reactions to tumor homografts -

Objectives - To ascertain the vascular phenomena associated with transplantation of homografts of tumor tissues, in order to assess the importance of vascular reactions in causing destruction of homografts.

Methods - Microscopic studies in living mice using transparent chamber methods, of the initial vascularization of first and second homografts of tumor tissue. These observations were correlated with biological tests of the duration of survival of the tumor cells in the first and second homografts.

Major Findings - First homografts were vascularized in from 1 to 4 days, and grew for 8 to 10 days before regressing. Second homografts made after regression of the first, failed to become vascularized or make any temporary growth; there was no evidence that vascular damage played a primary role in causing destruction of the tumors.

Significance -The findings are in accord with the concept that homograft reactions belong to the category of an active immunity. They fail to support the thesis that homografts are destroyed because of primary damage to blood vessels.

Future plans - This phase of the project has been completed.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

(b) Survival of homografts in immunized mice -

It was known from the work of Merwin and Hill (reference 5) that unvascularized homografts survived indefinitely in mice but were quickly destroyed if the host animal was immunized. The question arose as to the fate of such grafts when isolated from host tissue by porous membranes.

Objectives -

1. To devise and construct diffusion chambers in which cells would survive.

2. What would be the fate of homografts within diffusion chambers, isolated from host cells but nourished by extracellular fluid of the host?

3. As above, but when placed in immunized mice?

4. Items 1 and 2 above are parts of a more general question —Is destruction of homografts effected through contact of host cells with the "target" cells or through humoral factors.

Methods

1. Diffusion chambers were devised within which cells could be enclosed, returned to the skin or peritoneal cavity of the animal, and survive and proliferate although out of contact with host tissues.

2. Microscopic observations were made in living mice of the fate of homografts within diffusion chambers as compared to their fate beneath the membrane of the diffusion chamber, in contact with tissues of immunized mice.

Major Findings -

1. Procedures were devised for the construction of diffusion chambers which make use of membrane filters to separate grafted tissue from host cells and blood supply. Living cells within these chambers could be observed and photographed at high magnifications.

2. Isografts of various types of normal or malignant cells survived and grew for long periods of time within diffusion chambers, nourished by host fluids but physically isolated from host tissues.

3. Homografts of a normal or malignant tissue survived and grew within diffusion chambers under conditions in which similar grafts placed subcutaneously were rapidly destroyed.

Significance for Cancer Research -

1. An experimental method is described by means of which it is possible to obtain successful growth of tissue grafts in spite of their genetic incompatibility with the host animal.

2. The methods for diffusion chambers devised here have applicability to many problems of cancer biology such as transplantation of tissues, carcinogenesis, chemotherapy, host-tumor relationships.

Proposed course of project:

1. To utilize diffusion chambers in various problems as outlined in detail throughout this report.

(c) Interactions of heterografts with host.

Objectives -

Human tumors have been grown for limited periods of time in experimental animals subjected to total x-radiation or cortisone treatment; survival of heterografts has been attained also in such special sites as the anterior chamber of the eye and the brain of experimental animals. The recent development of diffusion chambers has provided a method for analysis of such questions as:

1. For how long will heterografts survive in diffusion chambers isolated from host tissues?

2. Does the host produce antibodies to heterografts enclosed in diffusion chambers?

3. Do host leucocytes participate in the destruction of heterografts?

4. Do heterograft cells undergo malignant alterations under conditions of the diffusion chamber?

Methods -

Heterografts were placed in diffusion chambers of mouse tumors into rats; human (Hela) strain cells from tissue culture into mice; and guinea pig sarcoma into mice. Observations were made either in vivo using transparent chamber methods, or as fixed and stained specimens after intraperitoneal implantation and later removal.

Major Findings -

In preliminary experiments mouse cells in diffusion chambers were destroyed within a few days in rats which had been previously immunized against mouse tissue, but survived for at least 7 days in control rats. Human (Hela strain) cells survived for at least 60 days in diffusion chambers placed intraperitoneally in mice.

Significance to Cancer Research -

Heterografts of human tumors in experimental animals are being used in other laboratories, in large scale studies of chemotherapeutic drugs. Fundamental studies of host-heterograft interaction will contribute to a rational assessment of this approach. If heterografts of human tumors will survive and grow in diffusion chambers, possible application to diagnostic or other problems of clinical cancer could be considered.

Future plans -

These preliminary experiments will be extended to determine if the host animal is immunized by the heterografts growing in diffusion chambers.

The improved diffusion chamber described above will be used to study the fate and behavior of heterografts through microscopic observation of the living cells.

PROJECT REPORT FORM (Cont'd)

10. NC-413
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 9728	3	2	5	2/3	2/3	1 1/3	
FY 1956	$ 4865	1	1	2	1/3	1/3	1/3	

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

5.NCI-4[I
SERIAL NO.

5. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Algire, G. H., Weaver, J. M., and Prehn, R. T.: Growth of Cells in vivo in diffusion chambers. I. Survival of homografts in immunized mice. J. Nat. Cancer Inst. 15: 493-507, 1954.

Prehn, R. T., Algire, G. H., and Weaver, J. M.: The diffusion chamber technique applied to the homograft resistance mechanism. J. Nat. Cancer Inst. 15: 509-517, 1954.

Algire, G. H.: Vascular reactions of Normal and Malignant Tissues in vivo. VII. Observations on vascular reactions in destruction of tumor homografts. J. Nat. Cancer Inst. 15: 483-491, 1954.

. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. Cellular Biology Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. ACI-414 — SERIAL NO

Mechanism of radiation protection by bone marrow.
PROJECT TITLE

Glenn H. Algire, Ruth Merwin, Charles Congdon
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION
Objectives -

Lorenz and coworkers have shown that injections of isologous or homologous bone marrow will protect mice against amounts of X-radiation which are lethal to uninjected animals. The mechanism of protection is not clear. One hypothesis is that the injected bone marrow cells survive and proliferate to take over the function of the marrow of the irradiated mice; another, that protection results from some as yet unidentified humoral factor. The objectives of these experiments are as follows:

a. To determine whether radiation protection can be brought about by bone marrow cells enclosed within diffusion chambers. A positive result would support the humoral theory; a negative result would be in accord with the cellular repopulation theory.

b. The second objective is to determine whether the bone marrow of mice protected against x-irradiation by injection of homologous bone marrow cells has been repopulated by homologous cells or by the animal's own cells.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Methods -

A. To determine the minimal effective number of marrow cells necessary for protection when given intraperitoneally; to include this number of cells in diffusion chambers (and multiples of this dosage) for intraperitoneal implantation into irradiated mice and to determine the effect on survival as compared with controls.

B. A test to identify the genetic strain of a given tissue is based on a previous finding that a nonvascularized graft of tissue in a host of a different strain is destroyed only when another graft of the same donor strain becomes vascularized.

Major Findings -

In preliminary experiments it has been found that marrow cells within diffusion chambers which are placed intraperitoenally. The dosage required for protection when marrow cells are injected directly into the peritoneal cavity has been found to be approximately 5,000,000 cells. In current experiments this dose and twice this cell population enclosed within diffusion chambers were placed intraperitoneally in previously irradiated mice. The results are not yet in.

PROJECT REPORT FORM (Cont'd)

M-414
SERIAL NO.

BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$4863	3	1	4	1/3	1/3	2/3	
FY 1956	$2,072	1		1	1/3		1/3	

BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Laboratory of Biology
LABORATORY OR BRANCH

3. Cellular Biology Section
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. Z01-CB-[illegible]
SERIAL NO

Effect of local irradiation with x-rays on neoplastic and normal tissues. Two problems are being studied, (a) The response of a radio-resistant tumor to irradiation, and (b) The effect of irradiation on the proliferation of endothelium in normal tissues.
PROJECT TITLE

Ruth M. Merwin
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION

Problem (a)

Objective:

In a previous paper the response of grafts of a radio-sensitive tumor to x-irradiation was studied in living mice using transparent chambers. We wish to make a similar study on a radio-resistant tumor.

Methods:

Because as reported previously several kinds of sarcomas when small enough to be observed in transparent chambers were found to be radio-sensitive instead of radio-resistant, larger growth were studied using a modified transparent chamber method for observing the grafts in living mice.

Major findings:

These larger growths are resistant to irradiation but detailed observations of changes within the tumors are more difficult. Observations have been made on the changes in the blood vessels of the surrounding normal tissues.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Significance:

Studies of the response of tumors to irradiation and their interaction with the surrounding tissues should prove useful in improving the treatment of tumors by irradiation.

Proposed course:

To continue these studies along the lines indicated.

Problem (b)

Objective:

The object of this study is to determine the effect of various doses of irradiation on the capacity of endothelium in normal tissues to produce new vessels.

Methods:

Within 6 hours after local irradiation with 400 to 1,500 roentgens, a stimulus for the growth of new capillaries was applied in the form of either a small burn or a loop of wire place through the skin.

Major findings:

(1) A dose of 500 r slowed the rate of revascularization of burned areas but had no effect on the time of vascularization of the coating of exudate that formed around tantalum wires.

(2) As the dose of irradiation was increased from 500 to 1,500 roentgens the rate of revascularization of burned areas decreased and the interval before capillaries began to invade the coating around wires increased.

(3) The new network of vessels in both types of injuries was sparser following doses of irradiation that exceeded 700 roentgens than it was following lower doses.

Significance:

A loss of the capacity to produce new vessels by the vessels in normal tissues affects the course of regrowth of tumors after irradiation. It may also be important in the injurious effects of irradiation on normal tissues, which some believe limits the dose of irradiation that can be given to a tumor. Also changes in irradiated blood vessels may be one of the factors that makes wounds in irradiated areas heal poorly.

Proposed course:

The studies described above were short term ones in which the stimulus for capillary growth was given within 6 hours after irradiation. It is planned to find out how long the capacity of the blood vessels to produce new vessels is altered.

PROJECT REPORT FORM (Cont'd)

NCI-415
SERIAL NO.

BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$4662	1	0	1	1	—	1	0
FY 1956	$3072	1	0	1	1/3	—	1/3	0

BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

15. ACI-415
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Ruth Merwin and Elizabeth L. Hill: Effect of local roentgen irradiation on the formation of new capillaries after injury. J. Nat. Cancer Inst: In press, Vol. 15, No. 4.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Laboratory of Biology
LABORATORY OR BRANCH

3. Cellular Biology Section
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. Z01-CB-04446
SERIAL NO.

6. Cellular mechanisms in the destruction of homografts
PROJECT TITLE

7. James M. Weaver, Glenn H. Algire
PRINCIPAL INVESTIGATOR(S)

8. Richmond T. Prehn
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Previous work indicated that mouse cells (considered as targets) survived in diffusion chambers in homologous mice previously immunized to the strain of origin of the target cells. There was evidence that host cells did not enter the chambers.

The objective of the experiments described here is to study the mechanism of the survival of homografts in diffusion chambers.

Methods: Target cells from mice were placed in diffusion chambers which were designed so as to exclude or to permit the entrance of host leukocytes and macrophages. The chambers were then implanted intraperitoneally in isologous, homologous, immunized homologous, heterologous (rats), or immunized heterologous hosts. In other experiments the effect of incorporating spleen cells with target cells in such chambers was studied.

Major Findings: Target cells in cell-impenetrable diffusion chambers survived in homologous and even in immunized homologous mice as well as they did in isologous mice. The fact that they did not survive in immunized rats as they did in nonimmunized rats indicated that such chambers were permeable to antibodies. However, other target cells did not survive more than about 10 days in immunized mice when these cells were implanted in more porous diffusion chambers which permitted host leukocytes and macrophages to enter. Control target cells in such chambers survived in isologous mice. Other control cells were sometimes killed in homologous hosts (not previously immunized) but not for 2 weeks or more. In a different type of experiment, target

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

cells were combined with washed spleen cells in cell-impenetrable diffusion chambers which were placed in hosts isologous to the target cells. The spleen cells were from mice which were isologous, homologous or homologous and previously immunized to the target cells. In the latter case the target cells were rapidly destroyed by the "immunized" spleen cells whereas the control, isologous spleen cells had no effect. Homologous but "nonimmunized" spleen cells had no early effect but sometimes destroyed the target cells later. There was evidence that the destruction of target cells both in cell-penetrable chambers placed in immunized mice and in cell-impenetrable chambers in which immunized spleen cells were also implanted was the result of contact with lymphocytes. It appeared that such contact was followed by the death of both the target cells and the lymphocytes and that finally these two types of cells died at a distance from the initial zone of contact.

Significance to Cancer Research: Homograft destruction has been previously demonstrated to be a process involving active immunization of the host but most attempts to demonstrate circulating antibodies have failed. The above results suggest that antibodies to homografts exist but are bound to lymphocytes.

Future Plans: "Diffusion table type" transparent chambers will be used to study details of the interaction between living target cells and immunized lymphocytes in vivo. The hypothesis that this interaction is complement dependent will be tested in vitro. The possibility that contact of target cells with immunized lymphocytes is followed by the release of antibodies which can destroy target cells at a distance and of antigens which can similarly destroy immunized lymphocytes will be tested in vivo. Diffusion chambers with two compartments separated by means of cell-impenetrable filters will be used. Target cells will be combined with immunized lymphocytes in one compartment and the condition of target cells simultaneously implanted alone or of immunized lymphocytes alone in the other compartment will be studied. Appropriate controls will be used to determine the specificity of any observed cytotoxic effects on cells in the latter compartment.

10. ac1-416
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 11,000	1	1/3	1 1/3	1	1/3	1 1/3	
FY 1956	$ 14,513	1	1	2	1	1	2	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None.

. NCI-411
SERIAL NO.

. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

A manuscript describing the above work entitled "The growth of cells in Vivo. II. The role of cells in the destruction of homografts in mice" by James M. Weaver, Blanche C. Algire, and Richmond T. Prehn was submitted to the Journal of the National Cancer Institute on November 10, 1954.

Algire, G. H., Weaver, J. M., and Prehn, R. T.: Growth of cells in vivo in diffusion chambers. I. Survival of homografts in irradiated mice. J. Nat. Cancer Inst. 15: 493-507, 1954.

Prehn, R. T., Weaver, J. M., and Algire G. H.: The diffusion-chamber technique applied to a study of the nature of homograft resistance. J. Nat. Cancer Inst. 15: 509-518, 1954.

. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None.

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Laboratory of Biology
LABORATORY OR BRANCH

General Biology Section
SECTION OR SERVICE

4.
LOCATION (IF OTHER THAN BETHESDA)

5. Z-1-GM-
SERIAL NO

"Investigations of the role of genes and their relationship to nongenetic factors in the development of cancer"
PROJECT TITLE

W. E. Heston W. E. Heston
PRINCIPAL INVESTIGATOR(S)

Delta Uphoff, Margaret K. Deringer, Thelma B. Dunn, Marvin Schneiderman
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objectives: To determine what genes are involved in controlling the occurrence of cancer, to localize their primary action and to determine how this action becomes manifest in increasing or decreasing the probability that a certain type of cancer will occur. To ascertain how the genes are related to non-genetic factors such as the milk agent, endocrine factors and physical and chemical carcinogens, and what is the final effect on the occurrence of tumors when combinations of these factors are introduced.

Methods employed: The mammary gland tumors and pulmonary tumors in the mouse are studied primarily. Well known inbred strains have been used together with the new V-ob (obese) strain received from the Jackson Laboratory and also a linkage strain carrying genes flexed-tail, shaker-2 and waved-2. Appropriate matings are made between strains of known but different genetic susceptibilities to these tumors and between the appropriate hybrids produced to observe the effects of the segregation of genes and of the alterations in genotypes. Foster nursing studies are introduced along with appropriate matings to observe the relationship of the genotype and the milk agent. Localization of gene action is approached through transplantation of tissues of one genotype into acceptable hosts of a different genotype and the observation of subsequent tumor development in the transplanted tissues. Carcinogens are introduced singly or in combination into animals of appropriate genotypes for determining the relationship between specific genes and the carcinogen injected in the induction of tumors. Graded doses of carcinogens have also been injected for dose response studies.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

ajor findings: Mammary gland tumors. Further analyses of a vast amount of data on the relationship of the genetic constitution to the milk agent suggests that strain C3H and strain C57BL may differ by but one gene in the control of the propagation and transmission of the milk agent, i.e. that there is but one gene controlling the survival of the virus.

The incidence of mammary gland tumors has been tabulated for breeding females of strain C3Hf (C3H without the agent.) for generations 6 through 17. Whereas in no generation has the incidence been as high as the 38 percent reported in breeding females in the first 5 generations the incidence has remained relatively high usually between 20 and 30 percent. This slight reduction in incidence may be owing to two factors 1) the females have not had quite so many litters as those of the first five generations had and thus the breeding factor is not so strong, and 2) the females of these later generation are not living so long as the earlier females lived, primarily because of a nephritis now occurring in many of them after a year of age. There is still, however, no evidence that the agent is involved in the etiology of these tumors. The tumors continue to occur scattered throughout the pedigree chart with no tendency for high tumor sublines to arise. The average tumor age in these C3Hf females has continued to be around 20 months.

The incidence of hepatomas in these C3Hf females has shown an increase in the females of the later generations ranging from 19 to 37 percent in the last four generations. This apparent increase may have been in part owing to the addition of a technical assistant to the staff in October 1951, who has checked the animals more closely so that more are autopsied after being found moribund and few now die in the cages. Data for males of these later generations are not yet available.

Other tumors occurring in significant incidences are ovarian tumors with an incidence as high as 8 percent in one generation, sub-cutaneous sarcomas ranging from 0 to 8 percent, reticulum-cell sarcomas ranging from 0 to 10 percent, hemangio-endotheliomas ranging from 0 to 5 percent and lung tumors ranging from 0 to 8 percent.

ulmonary tumors: In a test to determine whether or not the obese gene (ob) has any influence on lung tumors induced with dibenzanthracene there was found to be no difference in the occurrence of pulmonary tumors in the obese mice of the V-ob strain and in the nonobese littermates. These results are not in line with those for the A^y (lethal yellow obese) gene but with a more susceptible background the ob gene may increase lung tumors.

In a study of the relationship of the A^y gene to pulmonary tumors induced with urethan the animals bearing the A^y gene were more susceptible than those homozygous for non-agouti aa. These results are in line with those for spontaneous pulmonary tumors and those induced with methyl cholanthrene.

In the extension of the study of localization of gene action in the control of pulmonary tumors it has been shown that many tumors occur in genetically susceptible lung tissue whereas few occur in genetically resistant lung tissue although both were transplanted when yet foetal tissue into a common host. Thus the genetic control over lung tumor development is established at this early age so that it cannot be influenced by different genes of the host.

In further dose-response studies on induction of pulmonary tumors in strain A mice with dibenzanthracene a straight line relationship has been demonstrated between doses of .01 mg. and .5 mg. These results are compatible with a single event hypothesis for the neoplastic change. The point for the zero dose (controls), however, falls significantly above the extension of the line constructed for the other dosage points.

Other phases of both the mammary tumor work and the lung tumor work are not at stages appropriate for reporting at this time.

Significance: Genetic factors constitute a major set of factors in determining whether or not cancer will occur. Studies such as those reported above are therefore of significance in any program aimed at the eventual prevention of cancer through a knowledge of the etiology of cancer. Studies with the mouse serve as guides for studies on the etiology of cancer in man. The mammary tumors in the C3Hf females may be of particular significance in relation to breast cancer in women for having developed in the absence of the milk factor they may be more comparable to breast cancer in women than are other mouse mammary tumors resulting from the milk agent.

Proposed course of project: One of the projects to be set up in the coming year is that designed to determine more conclusively whether or not the relationship of the genes to the milk agent is that of a single gene. This will involve backcross studies with the females being tested by foster nursing appropriate test females. The possibility of a delay of one generation in the elimination of the agent is being taken into account in the plan of the experiment.

Further studies of linkage between pulmonary tumors induced with urethan and the genes shaker-2, waved-2, and flexed-tail are being undertaken. Burdette did not get an effect of flexed tail when urethan was used although we had noted such an effect with dibenzanthracene induced- and spontaneous tumors.

Obese males are being starved in an attempt to get them to breed with strain A females in order to produce backcross animals which should be more appropriate than the V-ob strain for testing the ob gene on pulmonary tumors.

The study of the effect of gold thioglucose on the occurrence of pulmonary tumors in the yellow (obese) and brown (nonobese) A/f F_1 hybrids is underway and should be completed during the coming year.

The study of the localization of the action of the lethal yellow gene on lung tumors by transplantation of lung tissue should be completed during the coming year.

Dose-response studies will be continued investigating the nature of the curve between zero dose and .01 mg. dibenzanthracene. A response curve is also going to be determined for a water soluble carcinogen — urethan.

Since in studies of induction of tumors with nitrogen mustard in A and C3H mice, "leukemias" have occurred although not in significant numbers we are now testing nitrogen mustard in C57BL mice for the induction of leukemia.

It is hoped that a large amount of completed work that has not been published owing to editorial duties can be prepared for publication following relief from the editorial duties on July 1, 1955.

PROJECT REPORT FORM (Cont'd)

10. NCI-417
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PAYDAYS DATA
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$44,980	2	5	7	2	5	7	
FY 1956	$45,140	2	5	7	2	5	7	

12. BUDGET ACTIVITY:

RESEARCH ☆ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

none

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

none

PROJECT REPORT FORM (Cont'd)

15. 121-417
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Localization of gene action in the causation of lung and mammary gland tumors in mice. (In Proc. Sym. on 25 years of Progress in Mammalian Genetics and Cancer.) J. Nat. Cancer Inst. 15: 775-791 (1954).

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. General Biology Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. ZIA CI-419 — SERIAL NO
6. Host-Tumor Relationships — PROJECT TITLE
7. Morris H. Barrett — PRINCIPAL INVESTIGATOR(S)
8. M. M. Springer — OTHER INVESTIGATORS

PROJECT DESCRIPTION

Host-Tumor Relationships

Objectives

A striking characteristic of multicellular organisms is that the morphology and behavior of each cell group or kind is strongly influenced by the other cells in its environment. There seems to be a sort of cellular ecology which maintains organization from earliest differentiation to death. Ample experimental evidence of such inter-cellular influences exists.

The most obvious qualitative difference between a cancer and normal tissue lies in the fact that normal tissues are restrained by mutual inter-cellular influences which result in controlled organization whereas malignant tissue is not subject, or is very little subject, to such restraint. The ultimate objective of this work is to learn the nature of such influences or restraints. The immediate objective is to learn ways for manipulating them experimentally. In other words, we would like to know the biological definition of a cancer or failing in that we would like to find means of modifying a cancer's behavior.

Methods

The basic procedure is transplantation of standard tumors into standard hosts and studying the effect of experimental variations on the behavior of either the tumor or the host. The disciplinary techniques involved are those of genetics, immunology, and experimental surgery.

Major Findings

The experiments can be explained more clearly if they are separated into those involving phenomena which are primarily in the host and those phenomena which are primarily in the tumor. The two areas overlap and complete separation is not possible.

Reactions of the Host

Most of this work might be called "Tumor Immunity". The underlying concepts and working hypotheses in this field were discussed in a monograph titled "The Nature of Tumor Immunity", which was presented in "A Symposium on the Origins of Drug Resistance" sponsored by the Office of Naval Research and the University of Pennsylvania in March 1954. Publication will be by Academic Press.

We previously reported that a host can be made immune to the implantation of a tumor by washed red cells or by red cell ghosts (membranes) but not when the cells or membranes have been broken up by relatively gentle procedures. There appeared to be a lability of the antigen which depended upon architectural integrity above the molecular level. The "antigen" also appears to be unusual in other ways. It is injured by exposure to formalin in dilutions as high as 0.01% and it is surprisingly thermolabile. The range of partial to complete inactivation varying from 42 to 44° C with periods of exposure of 15 to 60 minutes. Serum has a weak protective action. It is difficult to believe that the thermolability depends upon intracellular enzymes because ghosts washed with citrate buffer are also inactivated by 30 minutes' exposure to 44° C. Further efforts to learn the physicochemical characteristics of this unusual antigen will be made. This has not been published.

The incubation period associated with this antigen and immunity is also unusual. Repeated experiments showed that the immunity following a single injection of red cells rose to a high degree during the second week, as would be expected, but unexpectedly, it deteriorated during the third week and rose again in the fourth. A first guess might be that this fluctuation depended upon the way that the foreign red cells were retained in a subcutaneous depot for a time and then were released in a "flood". To check on that possibility the red cells of donors were tagged with Se^{75}. After such tagged cells were placed under the skin of the recipients, removal

from the depot was studied by appropriate counting procedures. It was found that 90% of the cells disappeared from the depot during 8 days and there was no count above background at 16 days. Thus the fluctuation depends upon something else; we do not know what. Incomplete experiments suggest that the immunity which can be induced by neoplastic cells does not show this freakish incubation curve. If that is true it will raise the question of whether we have hit upon an obscure biological difference between normal and neoplastic cells.

Others have said that the antigen which generates tumor immunity is an agglutinogen similar to those determining blood groups and shared by erythrocytes and other cells. This is a very important theoretical point and we have been attempting to gain more information regarding it. A large number of experiments have been completed but only major points will be mentioned. Our main conclusion is that current views in the field of "tissue immunity" probably are clouded by a too simple and too direct transfer of classical immunologic concepts and techniques. New techniques and viewpoints are needed. Erythrocytes that are capable of eliciting a high degree of "tumor immunity" usually do not generate hemagglutinins at the same time, and vice versa. When suitable systems are employed it can be shown that the "immunity antigen" is destroyed by mild sonic vibrations but the hemagglutinogen is not. Attempts to classify mouse red cells by classical techniques did not produce helpful results. A search for "partial" antibodies by various means including the Coombs test did not yield helpful results. When the new technique employing dextran was applied we, like others, could easily demonstrate hemagglutinins but they were not correlated with "tumor immunity antigens". In an attempt to clarify this Dr. C. Hoecker, of the University of Chile, spent some time in our laboratory. By the use of his special typing antisera we obtained useful correlations between our materials and those used in other laboratories but no help with the above problems. At present the immunity in our animals cannot be explained on the basis of the histocompatibility group of Snell nor the antigenic components described by Gorer and Hoecker. Most of this has not been published.

There were some indications that the size of the fragments of red cell membranes might influence the power to generate tumor immunity (Barrett and Hansen, Cancer Research 13: 269, 1953). Another experiment along these lines has been done. Rabbit antisera against either intact mouse cells or against the intracellular products of lysis will strongly agglutinate finely divided cell membranes and produce large aggregates. The ability to stimulate tumor immunity was not restored, however. This indicates that the previously observed effect of fragmentation does not depend upon mere aggregation.

There have been some weak indications that "virulent" tumors might sometimes produce their effects by way of a lowering of host resistance rather than directly by their intrinsic powers of growth. As a preliminary to study of that point, it was necessary to reinvestigate the effect of multiple inoculations of a tumor in order to decide if our experiments were influenced by Lewis' "breaking down of resistance", Greenstein's "constitutional factors peculiar to the tumor bearing animal", and Casey's "XYZ effect".

An extensive series of experiments showed that resistance can, indeed, be "broken through" by multiple inoculations but the effect clearly depends upon the "cachectic" state induced by large tumor masses. The effect is seen only in the continued presence of the first tumor and then only when it has reached a critical size. After the "breakdown" occurs it is nonspecific with respect to the genetic constitution of the tumor. A paper reporting these results is to appear in the December number of the J.N.C.I.

In the experiments just described it appeared that the bulk of the tumor was a critical factor. This raises a question as to whether in some cases a patient might benefit from incomplete removal of an "inoperable" tumor. Opposed to such a notion, in the opinion of some, is the possibility that an incision through malignant tissue might disseminate or "activate" a tumor which was not completely removed. With these things in mind animals were inoculated bilaterally with identical transplantable tumors. After a period of growth one tumor was divided by a cruciate incision while its mate was left undisturbed. Controls were also observed. There was no evidence (in this small series) that the host's condition was worsened by this procedure. In fact, it was bettered in two cases. Next two groups of mice were inoculated with a single tumor. After the tumors had grown to large size one group was set aside as controls and the tumors of the second group were incompletely excised. The experimental group survived significantly longer than the controls. In these mice the development of metastases appeared to be related to long duration, which is in keeping with the observations reported in the preceding paragraph. Thus far the experiments permit but do not prove the premise. There are many factors to be considered in this matter and much more work must be done. The experiments are now being extended to spontaneous tumors. Obtaining spontaneous tumors that are well matched is slow and possibly treacherous work. In the small amount done so far the observations are in keeping with what has been said already.

Reactions of the Tumor

We previously published reports that the power of a tumor to grow in standardized hosts could be altered experimentally by a process which we noncommittally called "adaptation". Three other laboratories (Hauschka in Philadelphia, Strong in Buffalo, and Klein in Stockholm) are now working on some aspect of this problem and have confirmed our observations in one way or another. We have repeated and confirmed our observations on specificity. Incident to this we have observed that one of the changes which may occur in the tumor is its tendency to grow for a considerable period and then regress further that this reaction may occur almost exclusively in one segregant of a genetic series. We have also

observed that when the tumor is "adapted" by exposure to several successive partly foreign hosts it has a tendency to reach a sort of plateau. The plateau may represent some major condition characteristic of "one factor" tumors. The reason for saying that is that finally in the course of over 150 transplant generations the stock tumor has come to resemble the various adapted sublines and all tend to behave as "one factor" tumors (they variously behaved as 4, 3, 2, and 1 factor tumors). Indications that one may go beyond this "one factor" condition may be seen in the fact that four times we have succeeded in growing the tumor in a resistant foreign strain. Attempts to extend this work depend upon obtaining another suitable tumor, which we are trying to do. Several attempts to distinguish between "stock" and "adapted" tumors by immunologic techniques, including Hoecker's typing sera, have failed.

We previously reported that the growth of a certain tumor in F_1 hybrid or resistant backcross hosts was enhanced by the presence of the mammary tumor milk agent in the host. This conclusion was based upon the presumption that other differences between the offspring of reciprocal matings would not be likely to explain the results. In cooperation with Dr. Deringer we have now completed a critical test of that presumption and have found it justified.

Significance

The field with which this work is concerned is so difficult and obscure as to make clear definitions and outlines almost impossible. Nevertheless there is a slowly growing body of evidence which indicates that there are interactions between tissues and between tissue and its host which involve critical factors in the phenomena of cancer, embryology, and transplantation of normal tissues. A defensible definition of cancer cannot be given today except in descriptive and biologic terms. The terms usually include things related to the experiments that we have been describing. The experiments may be significant in several ways both practical and theoretical.

The most important implication is that some of these results suggest progress toward understanding of what controls the growth of a cancer and how this differs from normal tissue. We have already seen that a tumor's power to overcome host resistance can be altered; its rate of growth, tendency to regress, and tendency to metastasize are all subject to experimental change. The host's resistance can be altered experimentally, not only between similar hosts but also in different tissues and sites in the same host. The changes in the tumor have already been detectable, antigenic, or serologic definition. One of the antigens which changed the most would be missed by current chemical methods. However one might consider a biologic description of the factors that sometimes cause or allow the tumors of man and animals to regress and that control metastasis. It has already been observed in this laboratory (and confirmed elsewhere) that such factors materially effect the outcome of many chemotherapeutic experiments.

The experiments on incomplete excision will have an obvious direct clinical application if they continue to turn out as at present. Palliation in some late cases of cancer might be possible by this method.

The experiments on "adaptation" are of importance in understanding transplantation of normal tissues as well as in cancer.

PROJECT REPORT FORM (Cont'd)

10. 101-418
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$26,073	2	2	4	1 2/3	2	3 2/3	0
FY 1956	$26,094	2	2	4	1 2/3	2	3 2/3	0

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

There is a complex theoretical interlocking between this work and the work of Drs. Algire, Grobstein, Law, Prehn, and Sanford.

PROJECT REPORT FORM (Cont'd)

15. ACJ-418
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Barrett, M. K.: The nature of tumor immunity. In a Symposium on the Origins of Drug Resistance. Academic Press. In press.

Barrett, M. K.: Resistance of mice to multiple inoculations of a transplantable tumor. J. Nat. Cancer Inst. 15: December 1954. In press.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. General Biology Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. ZO1-HI9 — SERIAL NO

6. Gastric Cancer Investigations — PROJECT TITLE

7. Morris K. Barrett — PRINCIPAL INVESTIGATOR(S)

8. None — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives

Efforts have been directed at attempts to find methods for producing or studying some of the supposedly etiologic factors in gastric cancer. The question to be answered here was, does otherwise normal gastric mucosa become abnormal when it is polypoid?

Methods

New techniques in experimental surgery have been developed to permit the observation of various influences on the normal mucosa of the dog's stomach.

Findings

By a plastic surgical procedure gastric polyps were constructed from normal mucosa in the stomachs of a series of dogs. No other abnormal condition or stimulus was applied. It was found that the mucosa of such polyps degenerated after 3 years and atrophic gastritis developed, but only on the tip of the polyp.

Significance

These experiments should influence the clinical management of polyps. If chronic gastritis is a precursor of gastric cancer, as is generally supposed, and normal mucosa on a polyp becomes atrophic after a time,

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

as these experiments show, then there is not much point in debating whether a polyp is malignant or benign before removing it.

Proposed Course

The colony of dogs has been closed but accumulated specimens in other experiments are yet to be studied. In addition we are trying to determine the feasibility of a series of experiments in rats.

PROJECT REPORT FORM (Cont'd)

10. MCI-419
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$3,108	1	0	1	1/3	0	1/3	0
FY 1956	$3,092	1	0	1	1/3	0	1/3	0

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None.

PROJECT REPORT FORM (Cont'd)

5. NCI-419
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Barrett, M. K.: Etiology of gastric cancer as a problem for experimentation. in Pack and McNeer: A textbook of gastric cancer. In press.

Barrett, M. K., Lefco, T., and Hansen, W. H.: Degenerative changes in the mucosa of artificial gastric polyps in dogs. Gastroenterology. In press.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. General Biology Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. ACI-420(a) — SERIAL NO

Studies on the Rous sarcoma virus. I. Further development of the virus-chicken test-system for general use in basic studies on carcinogenesis and other biological reactions.
PROJECT TITLE

W. Ray Bryan — W. Ray Bryan
PRINCIPAL INVESTIGATOR(S)

John B. Moloney, Dorothy Calnan
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objectives:

To bring about adequate control of the biological and experimental factors which cause excessive variations and interfere with precise systematic investigations involving the Rous sarcoma virus-chicken test-system (a potentially valuable system for crucial studies on carcinogenesis particularly from the view point of viral etiology).

Methods employed:

Groups of chickens are inoculated with graded doses of virus and dose-response curves are derived under various conditions appropriate to particular investigations. The dosage-response curves constitute the basic tools for the quantitative biological investigations. Completed studies reported last year (see publication listed under 16) made possible the preservation of standard samples of virus for quantitative potency reference. This development permitted the control of variations in potency which occur among different lots of virus and made possible the systematic investigation of sources of variation attributable to the host, which are now under way.

Major findings:

(a) Using standard preparations of virus it has been possible to study the variations among individual chickens as well as among different lots of chickens hatched at different times. These investigations have revealed an important source of heterogeneity among chickens of some lots (but not others) which appears to be associated with changes in the state of susceptibility of the host, after inoculation with the virus (i.e. during the progress of an experiment). A similar observation on another tumor virus (Beard et al) was attributed to the development of an "acquired resistance". If this can be proved, an important advance in knowledge of tumor virus action will have been made. The significance in relation to the present objective is that an important source of heterogeneity has been traced, and appropriate statistical procedures have been introduced for its control. The methods found to be applicable were those of Bliss and of Ipsen pertaining to the analysis of truncated normal distributions. The same precision of bioassay data obtained with the most accurately measurable animal viruses, such as those of vaccinia and influenza, can now be obtained with the Rous sarcoma virus (note. This represents a further increase in precision over that reported last year).

(b) Two graphic devices were prepared, and published (see under 16), which allow experimental biologists to make rough estimations, by simple procedures, of the numbers of test animals required for quantitative biological studies of various desired degrees of accuracy in various fields of biology (including the cancer and virus fields). Since costs and animal housing space are related to numbers of animals, the relative requirements in different fields of biology can also be estimated roughly.

Significance to cancer research:

Cancer is a dynamic biological process which can be investigated in the dynamic state only in living animals or in their tissues maintained in vitro. The present project represents a progressive effort to refine the techniques of cancer investigation in the living animal and to establish conditions under which the various variable factors which might interfere with experimentation, or with interpretation of results, might be kept under experimental or statistical control. From the economic standpoint, improvements in techniques of control during the past several years have resulted in a reduction by a factor of 8 of the number of unit biological observations required for a given degree of accuracy.

Proposed course of project:

Incidental to dose-response data accumulated routinely during the course of other systematic investigations, analytical studies will be made of the fluctuations from time to time of test animal sensitivity to the virus, and of host influence on the character of the dosage-response curve.

PROJECT REPORT FORM (Cont'd)

10. NCI-420 (a)
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$4863	1/3	1/3	2/3	1/3	1/3	2/3	
FY 1956	0	---	---	---	---	---	---	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM (Cont'd)

15 NCI-420(a)
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

W. R. Bryan, J. B. Maloney and D. Calnan.: Stable standard preparations of the Rouse sarcoma virus preserved by freezing and storage at low temperatures. J Nat. Cancer Inst. 15: 315-329 (1954).

W. R. Bryan: Relative precision of dose-response data in the virus and tumor fields as compared with that in certain other fields of biology. J. Nat. Cancer Inst. 15: 305-314 (1954).

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. General Biology Section — SECTION OR SERVICE
4. ____ — LOCATION (IF OTHER THAN BETHESDA)

NCI-5.420(b) — SERIAL NO

Studies on the Rous sarcoma virus. II. Purification of the virus.
PROJECT TITLE

John B. Moloney and W. Ray Bryan — W. Ray Bryan
PRINCIPAL INVESTIGATOR(S)

Dorothy Calnan
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objectives:

To obtain the Rous sarcoma virus in sufficient quantity, and in a sufficiently high state of purity, to permit its chemical and physical characterization, as well as investigations on its mode of action.

Methods employed:

(a) Enzymatic degredation of aggregated impurities from which the virus cannot be separated in appreciable amounts (i.e. in more than microgram quantities) by physical separation procedures, alone, (e.g. ultracentrifugation, ultrafiltration, adsorption). At the time of last year's report an increase in yield of freely dispersed particulates (in the virus size range) had been obtained by precipitating active extracts with protamine sulfate and treatment of the precipitate with the enzymes hyaluronidase, and trypsin. This approach was continued on an exploratory basis until the optimal conditions had been worked out, and only recently was activated as a full scale investigation.

(b) Physical separation methods. Once freely dispersed particulate fractions have been isolated from tumor tissue extracts, further fractionation of the particles of different sizes must be accomplished by physical procedures. Knowledge of the physical constants, density and sedimentation constant, were therefore required before adequate physical separation methods could be devised. These were determined last year in collaborative studies with Dr. H. Kahler (see his report, and publications under 16).

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Major findings:

By enzymatic methods together with differential centrifugation in an ultracentrifuge, the yields obtained in practical laboratory runs of freely dispersed particulates (of virus size) have been gradually increased to an order of magnitude of a few milligrams. On a unit of nitrogen basis, and in comparison with the starting crude extracts, the refined products have varied from about 100 to about 500 in their purification ratios. Since the relative proportions of virus and non virus particulates are as yet unknown, no statement can be made concerning the absolute purity. Nevertheless, virus preparations for use as standards of reference in quantitative biological experimentation (see project no. I) can now be prepared in workable quantities which are 5 to 25 times more pure on a unit of nitrogen basis than those previously available (see publications under project I).

Significance to cancer research:

The nature of the interactions between tumor viruses and other intracellular entities, or systems, which might be concerned in cancer formation cannot be investigated until the tumor viruses are available in relatively pure form. This is an attempt to provide one such tool for basic studies on carcinogenesis at the intracellular-system level.

Proposed course of project:

Efforts will be made to further increase yields of dispersed particulate fractions of this same degree of purity, and to further purify them by physical methods, taking advantage of the newly acquired knowledge of the physical properties, density, and sedimentation constant.

ONCH#20(b)
SERIAL NO.

1. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$14,594	1	1	2	1	1	2	
FY 1956	$7,297	1/2	1/2	1	1/2	1/2	1	

2. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]
REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

3. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

15. NCI-420 (L)
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

H. Kahler, W. R. Bryan, B. J. Lloyd and J. B. Moloney: The density of the Rous sarcoma virus. J. Nat. Cancer Inst. 15: 331-336 (1954).

H. Kahler, W. R. Bryan, B. J. Lloyd and J. B. Moloney: The sedimentation of the Rous sarcoma virus. J. Nat. Cancer Inst. 15: 337-339 (1954).

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Laboratory of Biology
LABORATORY OR BRANCH

3. General Biology Section
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. ACI 420(c)
SERIAL NO

Studies on the Rous sarcoma virus. III. The quantitative relationship between inducing dose and the amount of virus extractable from experimental tumors induce with graded doses of the virus.
PROJECT TITLE

W. Ray Bryan
PRINCIPAL INVESTIGATOR(S)

Dorothy Calnan and John B. Moloney
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objectives:

To determine whether there is a correlation between the dose of virus used to initiate a tumor and the amount of virus extractable from it. (Or, to answer the question: Is the chance of demonstrating virus in a cell-free extract of tumor tissue a function of the dose of virus by which the tumor was produced?)

This is considered to be a crucial definitive experiment designed to assess the plausibility of the "viral etiology theory", as a scientifically sound and potentially productive experimental approach to the problem of cancer causation in general.

Methods employed:

Prerequisite to the execution of this investigation (which because of technical necessity is extended for several months in time) was the availability of a stable standard preparation of Rous sarcoma virus (see project I) not only for inducing the initial tumors over a wide graded dose series, but also for use as a common standard of potency reference in later quantitative bioassays of cell free extracts of the various individual tumors.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Using a constant standard preparation of the virus, groups (ranging from 10 at high doses to several hundred at the lowest doses) were inoculated with relative doses ranging, in 10 fold steps, from a dilution of 10^{-1} through dilution 10^{-9}. The resulting tumors were removed from their hosts, oseptically, and stored in a CO_2 ice chest. Ten or more tumors are available at all dose levels from dilution 10^{-1} through dilution 10^{-6}, but only 3 tumors have been obtained thus far at dilution 10-7, and none at the highest dilutions (10^{-8} and 10^{-9}), i.e. the lowest doses. The tumors are being extracted and tested as rapidly as technically possible (the assay of each requires a minimum of 30 to 50 test-chickens and a period of 34 days). Before testing, each extract is freed of possible intact tumor cells by repeated centrifugation under conditions previously proven to be adequate for this purpose. (Filtration was not used because large quantities of virus are removed from suspension through adsorption on the filters). Over 40 tumors have been tested to date in quantitative bioassays involving approximately 2000 test-chickens (including controls).

Major findings:

The results are incomplete, but already it can be definitely stated that there is a highly significant correlation between the amounts of virus by which the experimental tumors were produced and the amounts which can be demonstrated in their cell-free extracts under constant testing conditions. Tumors produced with the highest doses (lowest dilutions) have uniformly yielded highly potent extracts, whereas those induced with the lowest doses (highest dilutions, e.g. 10^{-6} and 10^{-7}) have been uniformly relatively weak or, in a few instances, completely inactive. Furthermore, the tumors produced with intermediate doses have given cell-free extracts of intermediate potency.

Significance to cancer research:

In spite of the fact that certain special conditions are known under which virus cannot be demonstrated in tumors known to have been virus induced (e.g. the Shope papilloma produced in domestic rabbits (genus Oryctolagus) with virus extracted from papillomas of wild cotton tail rabbits (genus Sylvilagus), its natural host), it has been generally assumed by investigators in the cancer field that any tumor having a virus etiology should yield demonstrable virus on extraction. Corollary to this assumption has been the belief that failure to demonstrate virus in cell-free extracts of tumors arising spontaneously in nature, or under certain experimental conditions, is justification for the consideration of such "negative" tumors as being of non-viral origin.

The present findings show decisively that this is not a valid assumption, and that even with the most highly potent, and most rapidly acting, tumor virus known at the present time, reproducible experimental conditions can be found (involving relative virus dosage and relative

host susceptibility) under which tumors known to be virus induced will yield little or no virus on extraction. (The present tumors were tested under carefully worked out optimal conditions for this particular virus, which included their extraction with isotonic potassium citrate solution. Under the usual conditions more generally employed in testing for viruses, such as sodium chloride extraction and filtration through porcelain filters, it is probable that most of the tumors induced by the weakest doses would have been found to be "completely inactive".)

Since the incidence of tumors among experimental animals decreases with dose as the dose is lowered below a certain level (e.g. dilution 10^{-5} under present conditions), it is logical to assume that the "spontaneous" tumors which occur with extremely low frequency under natural conditions, would, if virus induced, represent the products of extremely low doses of virus. On a basis of the present findings, therefore, "spontaneous" tumors resulting from even as "virulent" a tumor producing virus as that of Rous sarcoma, would not be expected to yield detectable amounts of the inducing viral agent on extraction. The failure to demonstrate virus in tumors should not therefore be considered as contradicatory to an aggressive systematic approach to the problem of carcinogenesis in general from the viewpoint of a possible viral etiology.

Proposed course of project:

Several more months will be required for the completion of the present study, particularly its extension to extremely low doses which produce tumors with the very low frequencies (less than 0.1 per cent) observed under natural conditions (for sarcomas of the present type).

One of the first questions which presents itself for further investigation is whether the low, or nil, virus content of the low-dose tumors is due to: (a) failure of the virus to multiply to the same total extent as in high-dose tumors, and thus to reach concentration high enough to permit detection, or (b) production by the host of inhibitory or "masking" substances which neutralize the virus as rapidly as it is reproduced, when the initial invading doses are small. These alternatives are not mutually exclusive and both could be operative at the same time. Abundant evidence of inhibitory substances in some, but not in other, extracts of Rous sarcoma has been reported. One fowl sarcoma is known (tumor X of the Rockefeller Institute series) which, though transmissible by cell transplantation, failed to yield virus on extraction during its early history. Subsequent studies revealed that a virus could be recovered from "inactive" extracts of the tumors by precipitation in an ultracentrifuge. The supernatant was capable of reinactivating the recovered virus, however, if remixed with it. Attempts will be made to duplicate this phenomenon with extracts of low-dose tumors. If successful, conditions will be available for producing, at will, extracts containing either excess inhibitor or excess virus. Purification and study of these two factors, together with the interactions between them under controlled conditions, should throw light on the basic mechanisms of carcinogenesis, and, possibly, might suggest methods for slowing down or reversing neoplastic reactions. (See project IV, for a microbial product "Xerosin" (Groupe) which is capable of slowing down this neoplastic reaction to Rous sarcoma virus).

PROJECT REPORT FORM (Cont'd)

NCI-420(c)
SERIAL NO.

1. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$16,748	2/3	2	2 2/3	2/3	2	2 2/3	
FY 1956	$29,867	1 1/2	3	4 1/2	1 1/2	3	4 1/2	

2. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

3. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. General Biology Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-4420(L) — SERIAL NO

Studies on the Rous sarcoma virus. IV. The effect of the microbial product Xerosin (formerly known as "A.P.M") on tumors induced by the virus.
PROJECT TITLE

W. Ray Bryan
PRINCIPAL INVESTIGATOR(S)

Dorothy Calnan and J. B. Moloney (NCI), Dr. Vincent Groupe, Rutgers University.
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objectives:

To study the nature of the action of Xerosin which is capable of prolonging the latent period and slowing the growth rate of tumors induced with the Rous sarcoma virus (i.e. changing the tumors to a less malignant form).

Methods employed:

This investigation is still in the exploratory stage, see following "Summary of Project".

Summary of Project:

This exploratory investigation, being carried on in an informal collaboration with Dr. Vincent Groupe, Institute of Microbiology, Rutgers University, is not far enough advanced to merit the reporting of results at this time. The following summary is for the information of section, laboratory and branch chiefs, only, and is not intended for inclusion in a published annual report.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

As reported last year, Xerosin (APM) administered to chickens which had received relatively high doses of the Rous sarcoma virus caused a prolongation of the latent period (equivalent to a reduction in dose of 2 log intervals) and the transformation of some of the tumors (about 1/4) to a firm, slow growing type. The change in type was similar to that observed, less frequently, with very low doses of virus (e.g. ED 50 and below). No virus was recovered from one slow growing tumor carried through several transplants after arising in a Xerosin treated chicken. This suggested that Xerosin might be capable of "masking" the virus in vivo, or, if not directly, stimulating the chicken to do so through some mechanism already operative.

It is planned to resume studies on Xerosin in this regard as soon as possible, but, other background methods and information had to be worked out first. With the availability of stable standards preparations of the Rous sarcoma virus (see project I) a study of the occurrence of slow growing tumors at low doses, and of their virus content, was launched (see project III). The background information of the latter investigation is essential before the results obtained with Xerosin could be properly interpreted, and no new experiments involving Xerosin were started during the year. It is planned to resume them in about 3 months.

The significance to cancer research is that Xerosin appears to induce a condition in high-dose tumors which occurs naturally in low-dose tumors (see comments under project III), and that it may therefore constitute a valuable tool for attacking one of the crucial problems in virus cancer research (the "masking phenomenon").

NCI-420(d)

PROJECT REPORT FORM (Cont'd)

NCI-420(d)
SERIAL NO.

BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	0	--	--	--	--	--	--	
FY 1956	$ 4,865	1/3	1/3	2/3	1/3	1/3	2/3	

BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. General Biology Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-421 — SERIAL NO

Genetic Studies in Mice. A. Genetic Studies of Tumors. B. Genetic Study of a Mutant.
PROJECT TITLE

Margaret K. Deringer
PRINCIPAL INVESTIGATOR(S)

Dr. W. E. Heston
Dr. M. K. Barrett
OTHER INVESTIGATORS

PROJECT DESCRIPTION

jectives:

Genetic studies of tumors.

1. To study the effect of the injection of the mammary tumor agent into agent-free $C3H_f$ mice during the late stages of pregnancy at which time the agent has been reported to disappear from the blood of mice which carry the agent, and to see if the agent can be transferred through the uterine wall to the fetus (in cooperation with Dr. Heston).
2. To study the effect of the subcutaneous injection of o-aminoazotoluene on the production of hemangio-endotheliomas in strain HR mice. It has been observed that hemangio-endotheliomas developed spontaneously in 18% of a group of untreated strain HR mice. No other strain of mice has been described in which hemangio-endotheliomas occur frequently but they have been produced by subcutaneous injection of o-aminoazotoluene in strains BALB/c, A, C3H, and C57BL.
3. To produce a mammary tumor agent-free strain of mice from animals developed from transferred ova.

4. To determine whether a mammary tumor agent is present in strain HL (Bagg L) and in strain SWR (Swiss)(in cooperation with Dr. Heston).
5. To study the relationship of certain known genes (W' - dominant spotting, Ca -caracul, and Fu - fused tail) in mice to the production of pulmonary tumors induced by the injection of a hydrocarbon (in cooperation with Dr. Heston).

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

6. To produce special types of animals for collaborative experiments with Dr. M. K. Barrett (see Dr. Barrett's report for details).

Genetic study of a mutant.

1. To test a mutant, phenotypically-like waltzer which arose in strain A mice maintained in this laboratory (in cooperation with Dr. Heston).

Methods employed

1. Groups of strain C3Hf mice have been injected intraperitoneally with 0.5 cc. of a 5% cell free extract of a mammary gland tumor from a strain C3H mouse. The groups of animals were injected 1-3, 4-6, and 7-9 days before the birth of the young; immediately following the birth of the young; and when the first litters were weaned. Two groups, maintained as virgins, have been injected at 45 and 65 days respectively. Uninjected female mice have been allowed to produce one litter each and then have been retired at the time at which these litters were weaned. Approximately 200 female offspring of mothers injected before the birth of the young have been kept as virgins. The animals in this experiment will be maintained as long as possible and a study of the occurrence of mammary gland tumors and of other types of tumors will be made.
2. One hundred strain HR mice (equally distributed as to sex and as to the presence or absence of hair) have been injected with 100 mg. of o-aminoazotoluene dissolved in olive oil. The injections were started when the animals were 2 to 2½ months of age and were given in monthly doses of 10 mg. each. One hundred uninjected animals serve as controls. All of the animals are being observed for the development of tumors and of any unusual lesions.
3. Strain C3H and strain C57BL females are observed each morning for the presence of vaginal plugs - a sign that mating has occurred. Two or three days later the strain C3H females are killed and fertilized ova are obtained from the oviducts or uterus. These ova are transferred to the uterus of strain C57BL females and allowed to develop there.
4. Newborn strain C3Hf female mice are foster-nursed on strain BL and on strain SWR females. The foster-nursed animals are maintained as virgins and are observed for the development of mammary gland tumors.
5. The mice used in this experiment were offspring produced by mating F_1 hybrids of strains W'CaFu and A with strain 1194. The animals were injected intraperitoneally with 0.5 mg. of 1,2,5,6-dibenzanthracene at two months of age. They were autopsied at 10 months of age and were observed for the development of pulmonary tumors and of any unusual lesions.
1. Strain A males, showing the waltzing character were outcrossed to strain 1194 females. F_1, F_2, and backcross generations were produced and data were collected regarding the presence of the character in the different groups of animals.

Major findings.

1. In the group injected 1-3 days before the birth of the young, 9 of a group of 50 females have developed mammary gland tumors at an average age of 21 months; the non-tumorous animals died at an average age of 19.4 months. In the group injected 4-6 days before the birth of the young 11 of a group of 46 females have developed mammary gland tumors at an average age of 21.4 months; the non-tumorous animals died at an average age of 19.2 months. In the group injected 7-9 days before the birth of the young 4 of a group of 28 have developed mammary gland tumors at an average age of 16.0 months; the non-tumorous animals dying at an average age of 18.6 months. In the group of animals injected at the weaning of the first litter 5 of a group of 50 animals developed mammary gland tumors at an average age of 24.4 months; the non-tumorous animals died at an average

age of 19.8 months. Seven of 159 of the offspring of injected mothers developed mammary gland tumors at an average age of 19.3 months: the non-tumorous animals died at an average age of 20.4 months. Three of 57 of the females, retired after the weaning of their first litter, developed mammary gland tumors at an average age of 18.3 months; the non-tumorous animals died at an average age of 21.6 months. In the groups injected at 45 and 65 days, 4 of 40 females in the former and 2 of 42 females of the latter group developed mammary gland tumors at average ages of 16.2 and 19.0 months respectively; the non-tumorous animals died at average ages of 20.1 and 20.5 months.

2. In all of the animals of the injected group which have been autopsied, the incidence of hemangio-endotheliomas and of hepatomas has been increased over that of the untreated groups. Multiple tumors of both types have been observed in the injected animals as compared with the occurrence of single tumors in the untreated animals.

3. Seven successful transfers of fertilized ova have been made. Three lines of C3He mice have been started, one of which has been begun with a brother x sister mating.

4. Five of a total of 10 strain C3Hf females, foster-nursed by strain BL, have developed mammary gland tumors. The tumors have appeared at an average age of 14.4 months, the non-tumorous animals have died at an average age of 15.6 months. All of the C3Hf females, fostered-nursed on strain SWR, are still alive.

5. Seventy-five of 110 animals exhibiting dominant spotting developed pulmonary tumors following intraperitoneal injection of dibenzanthracene. Eighty-three of 129 animals without spotting developed pulmonary tumors. The difference between the two is not significant: $X^2 = .39$, P is between 0.5 and 0.7. Thirty of 49 animals with caracul coat and 128 of 190 animals with normal coat developed pulmonary tumors. The difference between them is not significant: $X^2 = .65$, P is between 0.3 and 0.5. Twenty-six of 49 animals with fused tail and 132 of 190 animals with normal tails developed pulmonary tumors. The difference between the two is significant; $X^2 = 4.7$, P is between 0.03 and 0.04.

1. In the (1194 x A) F_2 animals, 75 of a total of 634 animals (11.8%) exhibited the waltzing character and in the (1194 x A)-ABC animals, 97 of a total of 251 animals (38.6%) exhibited it. These results indicate that probably this character is due to a single recessive gene.

Significance to cancer research

A. It is already well established that the genetic constitution influences the development of tumors. All facets of this project will add more information to that already existing concerning the mode on inheritance of various types on tumors.

B. The study of the mutant may be of significance in providing another known character which will be of use in the linkage studies of factors influencing susceptibility to various types of tumors.

Proposed course of the project

1. This study will continue through 1955

2. All of the mice, with the exception of 15 haired females, have received the proposed dose of 100 mg. of o-aminoazotoluene. The remaining 15 animals will have received their total dose by April 1955. A large number of the experimental and the control animals have been autopsied. It is anticipated that this study will be completed in the second half of the coming year.

3. As soon as sufficient C3He animals are available, experiments which have been planned with Dr. Heston will be undertaken.
4. It is anticipated that this experiment will continue through 1955.
5. This experiment has been completed.
6. Special types of animals will continue to be produced for the experiments with Dr. Barrett.

B. 1. Further experiments, designed to established whether or not the character described as waltzer is due to a single recessive gene, are planned for 1955.

PROJECT REPORT FORM (Cont'd)

AC-421
SERIAL NO.

BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 14591	1	1	2	1	1	2	
FY 1956	$ 14593	1	1	2	1	1	2	

BUDGET ACTIVITY:

RESEARCH ☆ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Laboratory of Biology
LABORATORY OR BRANCH

3. General Biology Section
SECTION OR SERVICE

4. .
LOCATION (IF OTHER THAN BETHESDA)

5. ZO1-422
SERIAL NO

Irradiation Studies
PROJECT TITLE

Margaret K. Deringer
PRINCIPAL INVESTIGATOR(S)

Dr. Egon Lorenz (now deceased)
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objectives:

1. To ascertain whether the incidence of papillomas of the skin could be increased in strain HR mice following an exposure to 400 r X radiation within 12 hours after birth (in cooperation with Dr. Lorenz).
2. To study the breeding behavior of (C57L x A)F_1 hybrid mice following X radiation to the ovaries only, to the whole body, to the whole body with ovaries shielded, and to the whole body with ovaries and spleen shielded. To study further the mechanism of the production of ovarian tumors following irradiation (in cooperation with Dr. Lorenz).

Methods employed:

1. Hairless and haired mice of both sexes of the HR strain were exposed to a total dose of 400 r X radiation within 12 hours after birth. The animals were observed at weekly intervals during the course of the experiment. When they became moribund or died, autopsies were performed. Routine sections were made of certain organs and sections of any tumor or any unusual lesion were also made.

2. Groups of female (C57L x A) F_1 hybrid mice were exposed at two months of age to total doses of 400, 300, 200, 100, 50, 25, and 12.5 r X radiation to the ovaries only in one series and to the whole body in another. An additional group was exposed to a total dose of 500 r to the ovaries only. The exposed females were mated to untreated males immediately following irradiation. A group of untreated females, which were mated to untreated males at two months

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

of age, were maintained concurrently with the experimental animals. Groups of female (C57L x A) F_1 hybrid mice were exposed at two months of age to 400 r X radiation with ovaries surgically exteriorized and shielded by a lead shield and to 900 r X radiation with ovaries and spleen surgically exteriorized and shielded by a lead shield. Ten days following irradiation the animals were mated to untreated males. Groups of animals, in which the ovaries and the ovaries and spleen were surgically exteriorized and put into lead shields, were maintained as controls. The subsequent breeding behavior of all of these groups of mice was studied. Autopsies were performed on the majority of the experimental and control animals and material for histologic study was obtained.

Major findings:

1. A total dose of 400 r X radiation failed to increase the incidence of papillomas of the skin in strain HR mice but a decrease in the average age of appearance of the papillomas was noted. A few squamous cell carcinomas were observed in the irradiated hairless animals.

The number of ovarian tumors was increased in irradiated strain HR females as compared with that in the control females.

Damage to the ovaries and testes of the strain HR animals was the principal result of the exposure to a total dose of 400 r X radiation within 12 hours following birth.

2. Female (C57L x A) F_1 hybrid mice in which the ovaries only were exposed to total doses of 300 to 500 r produced one litter and were sterile thereafter. Litter size was reduced as compared with that of untreated mice.

Females in which the ovaries only were exposed to total doses of 50 to 200 r produced multiple litters of litter size somewhat less as compared with untreated mice.

Females receiving total doses of 100 to 400 r to the whole body produced only one litter, with reduced litter size except in the 100 r group, as compared with untreated mice.

Females receiving total doses of 12.5 r to 50 r to either site produced multiple litters of comparable size with the untreated animals in the 12.5 and 25 r groups but with reduced litter size as compared with the untreated animals in the 50 r groups.

The breeding behavior of animals receiving 400 r with the ovaries shielded was similar to that of the control group. In animals receiving 900 r with the ovaries and spleen shielded the average litter size was slightly reduced as compared with that of the controls.

Ovarian tumors occurred in all of the animals receiving total doses of 300 to 500 r to the ovaries only and total doses of 100 to 400 r to the whole body and also in some of the females receiving total doses of 50 to 200 r to the ovaries only and in some of those receiving a total dose of 50 r to the whole body.

The greatest effect of X radiation was noted when animals received irradiation to the whole body, a lesser effect when ovaries only were irradiated, and the least effect when the whole body was irradiated with the ovaries shielded. Although the greatest effect was obtained when the whole body was irradiated, it was necessary that the irradiation include the ovaries. No effect was apparent when the whole body excluding the ovaries was irradiated other than that which could be ascribed to the operation itself.

ignificance:

Radiation is employed in both the study of and the treatment of cancer. These studies aid in throwing light on the underlying mechanisms of its action.

roposed Course of Project:

This project has been completed in 1954.

PROJECT REPORT FORM (Cont'd)

10. M-422
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$3,108	1	0	1	1/3	-	1/3	0
FY 1956	0	-	-	-	-	-	-	0

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM (Cont'd)

15. ACI-422
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Deringer, M. K., and Lorenz, E.: Results of exposure of newborn strain HR mice to X radiation. (To be published in the Memorial Issue of the Journal of the National Cancer Institute, February 1955).

Deringer, M. K., Lorenz, E., and Uphoff, D. E.: Fertility and tumor development in (C57L x A) F_1 hybrid mice receiving X radiation to ovaries only, to whole body, and to whole body with ovaries shielded. (To be published in the Memorial Issue of the Journal of the National Cancer Institute, February 1955).

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. General Biology Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. ACI-423 — SERIAL NO

6. Developmental Physiology — PROJECT TITLE

7. Clifford Grobstein — PRINCIPAL INVESTIGATOR(S)

8. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Objectives: To increase understanding of the interaction of tissues and the components of rudiments in normal development as these relate to the differentiation of new cell types, and to apply information obtained to pathological problems, in particular to problems of tumorigenesis and malignancy.

B. Methods: Developing rudiments of mouse organs, e. g. kidney, salivary gland, and axial skeleton, are separated into their components and cultured in isolation and in various recombinations to determine the degree to which their development is interdependent and the nature of the interactive mechanisms involved. Development is observed in vitro and/or on reimplantation into the anterior chamber of the eye.

C. Major Findings: In the past year primary attention has been devoted to two interactive or inductive systems in vitro, embryonic spinal cord - kidney mesenchyme and embryonic spinal cord - somite mesoderm. These two systems differ in several characteristics, and it is hoped that in their detailed comparison clues to the nature of inductive mechanisms may be found. Advances have been made along the following lines:

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

a) Technique:

1) The tissue dish culture method developed last year has been improved and refined by modifications in the culture vessel and humidified incubator to give better optical conditions and greater reliability.

2) Methods have been developed for more reliable and convenient culturing of tissues on opposite sides of membrane filters, yielding higher frequencies of induction under conditions susceptible to more rigid experimental control.

b) New information:

1) Kidney system - It was demonstrated last year that embryonic central nervous tissue, like the kidney and salivary epithelial buds, can induce tubule formation in vitro when combined with embryonic kidney mesenchyme. Further studies have localized this activity in the dorsal two-thirds of the cord, the ventral-most region being completely inactive. Activity is present in 9 and 11-day cord, is weaker in the 13-day cord and is nearly absent in the 15-day cord. Four-day embryo chick spinal cord, at a stage comparable with that of the 9-day mouse embryo, can also induce tubules in mouse kidney mesenchyme, the activity being similarly localized in the dorsal region of the cord.

Much attention has been devoted to the interaction between spinal cord and kidney mesenchyme through membrane filters in an effort to characterize the filter-passing influence. When first reported last year this reaction was weak and variable, and the factors responsible for the variability have been extensively studied. Chief among them appear to be the porosity of the filter itself, and a number of technical procedures which effect the intimacy of contact between the two tissues and the filter. The reaction, under the conditions studied, seems to proceed best at porosities provided by the aerosol type filter and to be distinctly limited at the porosities of the hydrosol type filter. Thickness of the filter, up to 30 microns, does not appear to be limiting. On the other hand, separation of either tissue from the filter by even very small distances appears to completely eliminate the interaction. At porosities permitting strong inductions penetration of the filter by cellular processes occurs, but weaker, less regular inductions occur

at porosities which prevent cellular penetration visible with the optical microscope. Study is still in progress, but available data yet afford no evidence of freely diffusing molecules as the effective agency. Rather there is preliminary evidence of the involvement of a complex cohesive material including proteins and higher polysaccharides, possibly of mucopolysaccharide type. The role of the cellular processes is not yet fully evaluated.

The filter procedure permits interruption of the inductive process at any point by separation of the interacting tissues. Preliminary studies of this kind indicate that between 24 and 48 hours of exposure to spinal cord is required before kidney mesenchyme can continue to form tubules on separation. During this same period the first morphologically visible tubule rudiments appear. This clearly is the time of "determination" of new properties in the mesenchyme, and gives opportunity for control and detailed study of the events involved in cells and tissues as they alter in type.

2) Somite system - The segmented mesoderm or somites arranged on either side of the embryonic nervous system are the source of a number of cell types of the adult, e.g. cartilage, bone, and muscle. Recently, it was reported in lower vertebrates that the formation from these somites of vertebral cartilage is dependent on some stimulus from the spinal cord. It has now been shown, at a stage when somites alone produce no cartilage in vitro, somites combined with a fragment of spinal cord produce cartilage around the cord in arrangements which resemble those of normal development. A third mammalian inductive system thus is provided for study under controlled experimental conditions. Results on this system in comparison with the kidney show: (1) Dorsal cord is inactive for cartilage but active for kidney, whereas ventral cord is highly active for cartilage but inactive for kidney. It is clear that different activities of the cord are involved in the two inductions, and that they may have some reciprocal or mutually inhibitory relationship. (2) Cartilage-inducing activity, like that of kidney, is highest in 9-11 day embryo cord, declines by 13 days and is virtually absent by 15 days. (3) Cartilage induction is a longer term process than kidney tubule induction, first visible signs appearing at 4-5 days with the former, at 1-2 days with the latter. (4) Cartilage appears at a distance from the spinal cord rather than in immediate contact with it as in the case of kidney tubules. (5) Cartilage induction, like tubule induction, can take place through a membrane filter.

D. Significance to cancer research: The studies in progress are designed to increase understanding of the processes involved in the appearance of new tissue and cell properties in normal development. Involved are an external stimulus - in inductive processes exerted by neighboring tissues - a reorganization of the involved tissues and cells which becomes progressively more stable in the absence of the initiating stimulus, and the appearance of new cell types recognisable by their specific structure and metabolism. In the genesis of many tumors a similar course appears to be followed, some stimulus is applied from a source external to the involved tissue, the tissue shows changes usually referred to as pre-neoplastic which become progressively more stable, and at some point a new cell type appears which as it proliferates eventually is recognized macroscopically and clinically as a tumor. Tumorigenesis differs from processes in normal development in that in certain aspects it appears to reverse the normal course, and to represent a disruption of the interactions which limit the size and terminate the changes occurring in a normal structure.

It seems reasonable to assume that the interrelations set up in early stages, which have been demonstrated to be so important in controlling subsequent events, involve powerful mechanisms for controlling cell movement and arrangement of cellular masses, as well as of changes of cell type. The nature of these mechanisms is thus of considerable theoretical interest, and conceivably of practical interest as well. It may be significant that results of these first and still early studies of embryonic induction in mammals are to date suggesting the possible importance of the intercellular matrix or ground substance. The importance of this material in the adult has only relatively recently been recognized in studies of inflammation, infection and resistance. Its characteristic components, the glycoproteins, have been implicated in mechanisms of fertilization and virus penetration of cells, and immunochemistry has indicated the high specificity and variety of the compounds involved. These same materials are the indicators of externally induced hereditary transformations in Pneumococcus and other micro-organisms.

What this broad pattern means for cancer research is not easy to say. But there is hope that in the body of slowly developing knowledge in this field there may be clues to the control of a disease whose characteristic sign is the disruption of normal tissue relations by a cell of altered type.

E. Proposed course of project: It is proposed to continue the studies now in progress with particular emphasis on two points: 1) The nature of the mechanisms in the several in vitro inductive systems now available; 2) The nature of the changes occurring in the reacting tissue which initiate and stabilize its new properties.

Under the first heading the behavior of the several systems will be studied with filters of greater and lesser porosity to determine the correlation between presence and absence of filter penetration by cellular processes and the strength of inductive effects. It is hoped

that the higher resolution of the electron microscope can be applied to give more certain knowledge of the nature and extent of filter penetration by actual cytoplasm, since on this question rests the hypothesis of induction by infective transmission of particles directly from cell to cell. Attempts also will be made to obtain non-contact inductions by using restricted fluid volumes in which assumed diffusible inductors may be accumulated and concentrated with exchange allowed only via bridges following the principles of paper chromatography. Such procedures now appear feasible although many technical problems will need to be worked out. Finally, it is hoped that histochemical procedures, particularly those involving specific enzymatic digestion, may give useful information when applied to the "interzone" between the interacting tissues provided by the filter.

Under the second heading, the properties of the reacting tissue will be studied on removal of the inductor after varying periods of exposure. Determination will be made of the time at which the induced structures become stable, i.e. able to continue in their course in the absence of external stimulus, and the exact nature of the stabilizing change will be sought. Using available techniques of tissue disaggregation it should be possible to establish whether at the time of stabilization the individual cells are or are not already of altered type. In this way it is hoped to clarify the question whether induction is to be thought of as primarily a cellular transformation - - resembling induced hereditary alterations in micro-organisms - - or whether it is primarily a tissue or cell group reaction which only secondarily affects cell type. The problems here are, in the present state of knowledge, analogous withthose in tumorigenesis which have given rise to such concepts as "initiation", "promotion" and "autonomy". Both sets of problems may be encompassed under the more general phenomenon of the origin and stabilization of new properties in cellular populations.

MC1-423
SERIAL NO.

BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$34,448	2	3	5	2	3	5	
FY 1956	$34,564	2	3	5	2	3	5	

BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None.

IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not known.

PROJECT REPORT FORM (Cont'd)

15. NCI-423
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

1. In vitro induction of cartilage in mouse somite mesoderm by embryonic spinal cord (with G. Parker). Proc. Soc. Exper. Biol. & Med. 85: 477-481.

2. In vitro studies of cartilage induction in mouse somite mesoderm (with H. Holtzer). Submitted to J.Exp. Zool.

3. Tissue interaction in the morphogenesis of mouse embryonic rudiments in vitro. XIIIth Growth Symposium. Princeton University Press.

4. Tissue disaggregation in relation to determination and stability of cell type. In press, Ann. N. Y. Acad. Sci.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None.

PROJECT REPORT FORM

Cancer — 2. Biology
INSTITUTE — LABORATORY OR BRANCH

General Biology — 4. — 5. ZZI-424
SECTION OR SERVICE — LOCATION (IF OTHER THAN BETHESDA) — SERIAL NO

"Antigen-antibody-like" reactions of normal and cancerous cells.
PROJECT TITLE

Richmond T. Prehn
PRINCIPAL INVESTIGATOR(S)

Joan M. Main -- GS-7
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Object: The objective of this project is to study the physiology of certain "antigen-antibody-like" reactions of normal and malignant tissues and to determine the extent to which such reactions may be of importance to cancer biology.

Methods: "Antigen-antibody-like" reactions are allowed to take place under controlled experimental conditions (in vivo or in vitro) designed to modify the antigen, the antibody, or the union of the two. Such experimental conditions have included the use of:

a. Genetic alteration of the biologic system.
b. The use of vital staining.
c. The use of x-radiation.
d. The use of "diffusion chambers."

Major Finding: The major finding of this project during the past calendar year was the development of the "diffusion chamber" technique (in vivo tissue culture) and its application to the study of the social behavior of cells. By this technique foreign cells may be studied while bathed in the body fluids of a living animal, yet while isolated from direct contact with the animals own cells and tissues by a synthetic porous membrane. (Prehn, Weaver, and Algire).

Significance: "Antigen-antibody-like" reactions appear to be of fundamental importance to the biologic specificities involved in protein replication (necessary for growth and multiplication of cells), in hormone and enzyme reactions with substrates, in the penetration of specific cells by viruses, and in the specific social interactions of cells, as well as in the defense of the host against the growth of alien

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

cell types. A deeper understanding of the physiology of "antigen-antibody like" reactions in any of these contexts will lead to a deeper understanding of cancer biology.

As a by-product of this research, the "diffusion chamber technique", by making possible the growth of glandular cells within foreign hosts, may ultimately make possible a new, practical method of substitution therapy in endocrine-deficiency states.

Proposed course of Project:

It is believed that the project will be continued along the closely related courses indicated below:

a. A considerable portion of the next calendar year will be devoted to an investigation and exploitation of the preliminary discovery that high dosages of X-radiation plus administration of bone marrow may permanently alter or eliminate the reaction of mice to certain antigens (Main and Prehn).
b. The growth of endocrine tissues in "diffusion chambers" and their effects on the host will also be studied.
c. Possible methods of exploring the role of "antigen-antibody-like" reactions in cell adhesion will be investigated.
d. An investigation is being continued into the carcinogenic or co-carcinogenic properties of trypan blue and the possible relationship of such properties to the capacity of trypan blue to produce "R.E. blockade".
e. An investigation is also underway concerning the capacity (claimed by Foley) of MCA induced sarcomas to immunize inbred animals of the same strain as that in which the tumor was induced.

ZC1-424
SERIAL NO.

BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 23,367	1	3	4	1	2 2/3	3 2/3	
FY 1956	$ 25,249	1	3	4	1	3	4	

BUDGET ACTIVITY:

RESEARCH ☑ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Work related specifically to the development of the "diffusion chamber" technique was done in loose collaboration with Drs. Algire and Weaver of the N.C.I. - Lab. of Cellular Biology.

15. 421-424
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

1. Prehn, R. T., and Main, J. M.: Mobility and site of production of homograft resistance factor J.N.C.I. 14: 1277-1280, 1954.

2. Prehn, R. T., and Main, J. M.: A comparison between heterozygous and homozygous skin homografts. J.N.C.I. 15: 191-194, 1954.

3. Prehn, R. T., Weaver, J. M., and Algire, G. H.: The diffusion chamber technique applied to a study of the nature of homograft resistance. J.N.C.I. 15: Dec, 1954 (In press).

4. Algire, G. H., Prehn, R. T., and Weaver J. M.: Growth of cells in vivo in diffusion chambers. I. Survival of homografts in immunized mice. J.N.C.I. 15: Dec. 1954 (In press).

5. Main, J. M., and Prehn, R. T. Successful skin homografts after the administration of high dosage x-radiation and homologous bone marrow. J.N.C.I. Feb. 1955 (In press)

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

Cancer	2.	Biology		
INSTITUTE		LABORATORY OR BRANCH		
General Biology	4.		5.	Z01-425
SECTION OR SERVICE		LOCATION (IF OTHER THAN BETHESDA)		SERIAL NO

The biology of breast cancer in the mouse.

PROJECT TITLE

Richmond T. Prehn

PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION

Object: The objective of this project is to gain knowledge concerning the intraction of the multitudinous factors (including genetic, hormonal and viral) involved in the physiology of mouse mammary cancer.

Methods: The relative influence of the mammary tumor agent, tumor age, mouse strain and tumor morphology upon tumor distribution among the mammary glands of the mouse was determined by a statistical analysis of the data obtained, from the records of various animal colonies maintained at the N.C.I.

Major Finding: An uneven distribution of mammary tumors (increased anteriorly) among the glands occurred in the absence of the mammary tumor agent, whereas a comparatively even distribution occurred when the agent was present. The effect was largely a function of tumor age.

Significance: It is hoped that increased knowledge (~~Prehn and Main~~) of the physiology of breast cancer in mice will lead to an increased understanding of the physiology of human cancer.

Proposed course of Project: The project will probably be temporarily discontinued during the coming calendar year, owing to the competition of other projects for the available working time.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

10. E4-425
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$1755	1	3	4	0	1/3	1/3	
FY 1956	0	1	3	4	0	0	0	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM (Cont'd)

41-425
SERIAL NO.

PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

1. Prehn, R. T., Main, J. M., and Schneiderman M.: Factors influencing tumor distribution among the mammary glands of the mouse. J.N.C.I. 14: 895-904, 1954.

HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. General Biology Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. ZO1-426 — SERIAL NO

"Effects of the A^y and ob genes on mouse physiology."

PROJECT TITLE

George L. Wolff (Dr.)

PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objectives: The objective of this project is to determine the primary and pleiotropic effects of the A^y and ob genes on mouse physiology especially as related to the high incidence of lung cancer in A^y-bearing mice and as related to the hormone balance in mice carrying the A^y and/or the ob genes.

Methods Employed: To study the effects of the A^y and ob genes on the identical genetic background an attempt is being made to develop an inbred strain carrying both genes.

The anaerobic glycolysis of the kidneys (in the presence or absence of insulin and/or testosterone) of male and female Ob- and obob mice at various ages is determined by means of a Warburg respirometer apparatus.

The anaerobic glycolysis of Harding-Passey mouse melanoma (in the presence or absence of insulin and/or testosterone) after transplantation to normal and gonadectomized, male and female, Ob- and obob mice is being studied by the same method.

Major findings: The major finding of this project since its inception on July 1, 1954 has been the determination of a statistically significant difference in the rate of an anaerobic glycolysis between Ob- and obob mice at less than 40 days of age.

It has also been observed that Harding-Passey melanomas transplanted to obob mice have a consistently lower rate of anaerobic glycolysis than after transplantation to Ob- mice. higher

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Significance to Cancer Research: In order to obtain an understanding of cancer, it is necessary to learn how genes control the balance of hormones in normal and tumor cells so that any differences between the latter, not due to genic control, may be exploited in the treatment of neoplasms.

To explain the high incidence of lung tumors in mice bearing the A^y gene as opposed to a low incidence in littermates not carrying this gene it is necessary to know which specific metabolic reaction is under the control of this gene. The solution of this problem might ultimately provide a method of preventing lung tumors and of treating lung tumors chemotherapeutically.

Proposed Course of Project: The studies of the anaerobic glycolysis on Harding-Passey melanomas transplanted to Ob- and obob, mice will be continued and, as soon as the melanomas becomes adapted to the Y_{257} mouse strain, this investigation will be extended to include A^y- and aa male and female mice, both normal and gonadectomized. If the attempt to combine the A^y and ob genes in one strain should prove successful, similar metabolic studies will be conducted on A^y-obob animals to determine any possible interaction effects.

As soon as sufficient A^y- and aa animals are available, experiments designed to elucidate metabolic differences between the lungs of the two genotypes will be started.

PROJECT REPORT FORM (Cont'd)

10. NC-426
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$11,080	2	2	4	1	1/3	1-1/3	
FY 1956	0	-	-	-	-	-	-	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Biology — LABORATORY OR BRANCH
3. Leukemia Studies Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-427 — SERIAL NO

Studies in Experimental Leukemia.
PROJECT TITLE

Drs. Law, Potter, Reaume, Shacter and Stewart.
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION

The work relating to investigations in experimental leukemia falls into 2 major headings: A) studies of the etiologic factors involved in the induction of leukemia and B) mechanisms involved in the antileukemic activity of certain selected antimetabolites, e. g., antifolic and antipurine compounds.

A) Studies of the etiologic factors involved in the induction of leukemia.

1. The role of the thymus in leukemia. (Studies with Dr. M. Potter) These studies are designed to determine the precise role of thymic tissues, in relation to the gene, possible non-cellular etiologic factors and x-radiation, carcinogens, etc.

Thymectomy strikingly reduces the incidence of spontaneous x-ray -and carcinogen-induced leukemia in several strains of mice. Substitutions of thymic fragments in thymectomized mice (DBA, C57BL and C3H AKR F_1 hybrids restores the incidence characteristic of the test mice. In hybrid mice, transfer of thymic fragments from the low-leukemic parent does not result in the transplant becoming leukemic. Splenic fragments are known to be ineffective. Likewise, homogenates and filtrates of AKR thymuses are not effective in inducing leukemia in C3H AKR F_1 test mice.

The following projects are proposed relating to this aspect of the problem of etiology:

1. The effect of transplanted thymic tissue in mice following exposure to x-irradiation. Thymic tissues from C57BL/KA and A/HE mice

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

have been transplanted to F_1 hybrid mice of these 2 strains following a) thymectomy, b) total body irradiation (225 r x 4), and c) thymectomy + total body irradiation. We have confirmed Kaplan's observation that irradiated, thymectomized mice receiving thymic transplants show lymphoid neoplasms (the thymic graft becomes leukemic). In fact, thymic fragments from 1 to 5 day old $C57BL/_{KA}$ mice have become leukemic 1 month after irradiation. It is proposed to study particularly using this experimental model:

a. The influence of both C57BL and A thymuses in thymectomized, irradiated F_1 mice.

b. The influence of age of the thymic graft.

c. The pathogenesis of the process. Does this in fact represent a case of carcinogenesis of thymic tissue after the removal of x-irradiation (indicating an indirect effect of x-rays)? Transplantation of these induced leukemias into C57BL, A and F_1 hybrid mice will be made in an attempt to answer this question.

To date 2 large experimental groups of C57BL x A F_1 mice are under observation. The influence of A strain grafts and of age remain to be investigated.

2. Role of thymus in spontaneous leukemias.

The F_1 hybrid of the cross C_3H_f (low-leukemic x AKR (high leukemic) develops a high incidence of leukemia (60 percent at 14 months). It has been shown in this laboratory that thymic grafts of AKR origin (but not C_3H_f) in non-thymectomized hosts, becomes neoplastic and that the thymic graft is now composed of host F_1 tissue. This study is being continued to include the following projects:

a. The influence of thymic grafts (AKR) in thymectomized F_1 mice. It has not been determined in this experimental model whether or not an intact test animal is necessary for leukemogenesis in the transplanted thymic fragment.

b. A study of test mice of the reciprocal cross, AKR x C_3H_f, including the incidence in this hybrid, theeffect of thymectomy and the influence of AKR - C_3H_f thymuses.

c. These specific F_1 hybrid mice (AKR x C_3H_f and C_3H_f x AKR) will be used to study the pathogenesis of the change from normal to leukemic:

1. Since it appears that the reticular tissue of the thymus is important in inducing leukemia in the round cells, it is proposed to study the interdependence of these tissues by varying the age of the donor graft (1-7 days, 30 days and 120-150 days) and the age of the recipient hybrid (like ages). It is also proposed to study

the precise time of change from a normal to leukemic cell by bioassaying the transplanted thymic tissue in F_1 mice and also through the use of C57BL thymuses in irradiated C57BL x A F_1 hybrids. As mentioned earlier, it appears that the neoplastic change takes place as early as 4 weeks after irradiation.

To date, the group under 2a. has been set up and a start has been made in the production of mice for 2b.

3. The role of cell-free materials in the induction of leukemia and parotid gland neoplasms.

We have been unable to confirm the work of Gross indicating a cell-free material is obtained from high-leukemic tissues which induces leukemia at a strikingly early age in C_3H_f test mice. The following results are obtained to date:

	No. Mice	Leukemia	Parotid Tumors
Exptl.	283	13 (4.5%) at 17 mos.	21 (7.4%) at 11 mos.
Control.	161	5 (3.1%) at 21 mos.	none

We have now under observation 280 C_3H_f/AKR F_1 test mice given filtrates and extracts of high leukemic tissues from AKR, C58 and $C_3H/_{Figge}$ mice. It is of interest to note that parotid gland neoplasms have been induced in those mice through the use of material from all 3 strains. Though the incidence of leukemia at the 12 month period is nearly identical in experimental and control groups (16% and 13%), 1/3 of the leukemias have appeared at from 4 to 9 months, before any were observed among controls.

The following ther interesting observations were obtained:

a. In C_3H_f x $C_3H/_{Figge}$ F_1 test mice only parotid gland neoplasms and subcutaneous growths are obtained (25%). In nearly every case these subcutaneous growths appeared in mice bearing bilateral parotid gland neoplasms. In several cases the morphology of these subcutaneous growths closely paralleled that of the parotid gland neoplasm.

b. Observations on 5 families, through the F_3 generation (obtained from females having parotid gland neoplasms) indicate that the agent concerned is not transmitted by way of the cytoplasm, across the placenta or through the mother's milk.

c. From filtrates of one parotid gland neoplasm carried in serial passage, we have been able to induce 25% parotid gland neoplasms in C_3H_f test mice at the G2, G3 and G8 transfers. Serial passage of the agent is being attempted.

The following proposed projects are either in progress or will be initiated soon:

a. Morphologic study of parotid gland neoplasms in comparison with those induced by carcinogens and those occurring spontaneously (Dr. Thelma Dunn, Laboratory of Pathology).

b. Study of the mechanisms involved in the induction of parotid-like neoplasms in the subcutaneous connective tissues of C_3H_f x $C_3H/_{Figge}$ F_1 mice (employing $C_3H/_{Figge}$ leukemias).

c. A study of the incidence of leukemia and morphologic forms in a reportedly high-leukemic subline of C3H mice obtained from Dr. Frank Figge of the University of Maryland Medical School. To date 20 percent leukemia and several parotid gland neoplasms have been observed in our colony. Crosses have been made reciprocally with STOLI mice to determine a possible genetic basis for the increase in leukemia within this strain.

d. Since it has been suggested that subline differences among the test mice employed (C3H) may account for difficulties in obtaining induced leukemias from filtrates of high-leukemic tissues (AKR), it is proposed to use the following test mice to further study the possibility of a cell-free material in spontaneous leukemias:

$C_3H/_{Bittner}$ subline from Gross

$C_3H_f/_{Bittner}$ subline from Gross

$C_3H/_{Bittner}$ subline from Bittner.

e. The major effort in this particular project will be directed along lines of study of the parotid tumor agent. (Studies with Dr. M. Potter)

In order to evaluate certain features of the problem standard inocula have been prepared and stored at -60°C (from a transplantable parotid gland neoplasm and from induced neoplasms) so that repeated studies can be made. Certain evaluations encompassing the following features have now been started:

1. Inoculations of mice of different inbred strains
2. Effects of different types of filtration
3. Ability to retain activity after various periods of storage at -60°C
4. Activity following lyophilization
5. Titration studies of the standard inoculum.

f. In cooperation with Dr. W. Rowe of the Laboratory of Infectious Diseases, a study has been initiated of the effect of this agent in other animal species, its relation to other known viruses of the mouse, and a determination of possible cytopathogenic properties in tissue cultures.

g. The following progress report and projected program is submitted by Dr. Sarah E. Stewart:

1. In previous work it was found that parotid gland neoplasms, a tumor not previously described, could be produced in some C3H mice by inoculating newborn mice with extracts or filtrates prepared from AKR leukemic tissues, and that the C3H mice were resistant to the leukemogenic activity reported by Gross for these filtrates (Proc. Soc. Exper. Biol. and Med. 76: 27-32, 1951).

2. An adrenal gland medullary tumor of a type not previously described was observed in some C3H mice which had been inoculated as newborn with extracts or filtrates of a transplanted C3H leukemia. Many of the mice having these tumors, which occurred bilaterally in some and which metastasized widely, also had either unilateral or bilateral parotid gland tumors.

3. A leukemogenic agent was demonstrated in filtrates prepared from AKR leukemic tissues by inoculating newborn (C_3H_f x AKR)F_1 hybrid mice with these cell-free preparations. Leukemias specific for the hybrid mice resulted in 60 percent of the inoculated mice in a 14 month observation period. These begin to appear in mice only 10 weeks old. Control mice under observation developed a 23 percent incidence leukemia in the same period and the earliest one appeared in a 9 month old mouse. These experiments were repeated using a different substrain of the C_3H_f (Dr. Jay) for mating, the other C_3H_f mice were obtained from Dr. Law, but both were originally Dr. Andervont's substrain. Again early leukemias were observed but some of the mice instead of leukemia developed early parotid tumors, adrenal medullary tumors and, in 4 instances, mammary tumors which appeared in mice 3 months old. Fifty-four percent of the inoculated mice have developed neoplasms in a 9 month observation period.

4. A filterable agent has been propagated from filtrates prepared from 2 different transplanted C3H leukemias. This was passed serially through 15 generations and after the 5th and 8th serial passage, when inoculated into newborn mice, was found to produce parotid gland neoplasms in some surviving mice 5 to 12 months old, and in one instance an early adrenal gland medullary tumor.

The above work was completed July 1, 1954 while stationed in Baltimore, Maryland.

Work in progress and proposed work:

1. Studies are in progress to determine if adrenal gland neoplasms can be produced by inoculating newborn mice with cell-free extracts prepared from 3 different transplanted adrenal medullary tumors which results in experimental mice (see above). Strain C3H mice and $(C3H_f \times AKR)F_1$ hybrid mice which had been inoculated as newborn with such preparations are being gonad-ectomized. Others of the same group are being held as controls without gonadectomies.

2. Studies are in progress to determine if estrogens have an adjuvant effect on the leukemogenic activity of leukemic cell-free extracts. Strain C3H mice were inoculated as newborn with leukemic cell-free extracts or filtrates and when 2-4 months old were given extrogen pellets (20%) to see if their susceptibility could be increased.

3. Work is in progress to attempt identifying the leukemia-producing agent in mouse leukemic tissue; also the agent that produces the adrenal gland and parotid gland neoplasms.

4. To repeat Gross' work using C3H mice (Bittner substrain). The mice have been ordered.

5. Newborn C3H mice have been inoculated with mammary tumor agent to determine if the incubation period for the appearance of mammary tumors can be decreased.

The effects of various endocrine imbalances on methylcholanthrene-induced leukemia in DBA mice (Dr. M. Potter)

Gonadectomy has been reported to restore leukemogenic properties to an otherwise refractory thymus in old DBA strain mice (6 months of age). Hybridization of DBA with CE strain has produced an F_1 hybrid with unusual endometrial pathology. Thyroidectomy doses of I_{131} have been reported to induce pituitary tumors in DBA mice. The effect of these changes (gonadectomy, hybridization with CE and I_{131}) on leukemogenesis is being studied.

a. Gonadectomy

DBA mice are being gonadectomized at different age levels: 1-7 days, 30-40 days, and 4-6 months. At age 6 months each group with controls will be painted with MCA.

Induced leukemia (thymic lymphomas) will be transplanted to gonadectomized and intact DBA mice.

b. Hybridization

Reciprocal crosses (DBA_f x CE and CE x DBA_f) have been made and will be studied for incidence. The influence of gonadectomy performed at 1-7 days on leukemogenesis and transplantability will be studied in CE x DBA_f mice.

Similar experiments have been started employing still another strain showing evidence of endocrine imbalance, the NH strain.

c. Data will be given in thyroidectomizing dosage levels to DBA and the above hybrids to determine such an influence on leukemogenesis.

5. A study of granulocytic leukemias in R_f strain mice.

Through the courtesy of Dr. Jacob Furth we are now establishing a colony of R_f mice. These are reported to show an incidence of 30-40 percent granulocytic leukemia. We are ready to initiate the following program when animals become available:

a. Study of etiologic factors in granulocytic leukemias

1. Role of thymus
2. Response of R_f mice to x-rays, carcinogens and estrogens
3. Influence of protective factors such as MRF and bone marrow on induction of granulocytic leukemias
4. A study of the transplantability of granulocytic leukemias. Furth has suggested that many of those are really "dependent" lymphomas.
5. Morphologic study of granulocytic leukemias.

6. A study of certain protective factors in induction of lymphocytic leukemias

a. Maternal resistance factor.

Differences in the incidence of leukemia appear in F_1 hybrids in reciprocal crosses between high-and low-leukemic strains of mice. No evidence has appeared of a specific leukemia-inducingfactor. On the contrary a striking and specific resistance to leukemia has been shown to be contributed by certain low-leukemic mothers, so that in special cases nearly complete resistance to leukemia results, completely overriding the genetic potentialities of the test mice employed. It is the objective of this study to explore the characteristics of this maternal resistance factor (MRF).

The following additional information has been gained concerning MRF:

1. Confirmative evidence has been obtained to show that low-leukemic C3H mice do not transfer MRF. Crosses with AKR mice show same results as previous crossed with high-leukemic C58.

2. MRF is contributed through the milk of STOLI mice suppressing leukemia in AKR mice. Thus, the genotype of the high-leukemic strain seems relatively unimportant. Leukemia in C58 mice has been shown to be suppressed (MacDowell).

3. Other factors are operative, as shown in foster-nursing experiments, which modify the response of AKR and C58 mice. For example, in some cases old STOLI mothers (1 year or older) in specific instances do not suppress leukemia.

4. Attempts to pass on MRF in successive generations of C58 mice have been unsuccessful.

5. Foster-nursing alone by NH low-leukemic mice contributes MRF to C58 and AKR test mice.

The following work is in progress or will be initiated shortly:

1. Attempts to determine the mechanism of action of MRF -- whether it operates in repressing cells already transformed to leukemic cells or modifies the conditions under which changes to the neoplastic state occur. Periodic bioassays of blood and tissues of AKR mice from 5 months onward will be made.

2. Crosses of STOLI with C57BL and DBA will be made to determine if MRF represses x-ray induced and carcinogen-induced leukemia. Also, foster-nursing of C57BL by old and young STOLI mothers will be done prior to x-raying at varying dose levels -- 225 r, 225 r x 2, and 225 r x 4.

b. Bone-marrow repressive factor.

In cooperation with Doctors Lorenz and Congdon, Laboratory of Biophysics, it has been found that intact bone marrow cells from C3H mice (low-leukemic) repress leukemia in C_3H_f x AKR F_1 mice, while cells from 1 month AKR induce leukemia earlier. Such protection has been observed also among F_1 mice untreated. Two more series have been done on the effect of C3H bone marrow on the spontaneous incidence of leukemia in these F_1 test mice. In one series done by Dr. Congdon, this repressive effect has again been found. In the series done in our laboratory, using different experimental design, no effects were noted.

The following plan, partly in effect, is proposed as an extension of this work:

1. Study of specificity of action. Bone marrow from other high-leukemic strains: C58, PL and C3H/Figge and other low-leukemic strains NH, C57BL have been inoculated into the test mice, $C3H_f$ x AKR F_1 hybrids.

2. Influence of the age of recipient mice. Studies on inoculations into 1 month, 4 month, and 6 month F_1 hybrids have been initiated. It is proposed to inoculate C3H cells into 1-3 day F_1 and AKR mice.

3. Another cross C58 x NH will be made to study the influence of C58 and NH bone marrow cells in these F_1 test mice to determine if the phenomena are specific to the mice originally used.

4. Once threshold and maximal dosage levels of intact bone marrow cells have been established experimentally, a study will be commenced on the influence of bone marrow cells on the induction of other than lymphocytic lymphomas: granulocytic leukemias in R_f mice and reticulum cell sarcoma in irradiated C57BL x DBA F_1 hybrids.

B) A study of mechanisms involved in the antileukemic effects of certain selected antimetabolites.

Through the use of specific sublines of leukemia which have developed resistance or dependence to antimetabolites a study of the mechanisms involved in resistance phenomena, and a study of the mechanism of action of antileukemic agents, is being pursued.

The following transformed leukemia lines are available for such work: L1210 sensitive, L1210 (A-methopterin dependent), L1210 (8-Azaguanine resistant), L1210 (8-Azaguanine dependent), L1210 (6-Mercaptopurine resistant), L1210 (Thioguanine dependent); L4946 sensitive and L4946 antifolic resistant; L5178 sensitive and 2,6 diaminopurine resistant.

These lines have also been developed into ascites tumors which will facilitate biochemical studies and studies on growth differences, etc.

The major emphasis on work in this field involves the following projects already in progress, or to be initiated shortly:

1. Preliminary evidence suggests a difference in nucleic acid metabolism between those lines resistant to antifolics and those

resistant to such antipurines as: 6-Mercaptopurine, 8-Azaguanine and thioguanine. A study in cooperation with Dr. Howard Skipper, Southern Research Institute, Birmingham, Alabama is now in progress studying the incorporation of labelled formate, adenine and 2,6-diamino-purine into the various L1210 sensitive and resistant lines of leukemia.

2. A comparison of the incorporation of 8-Azaguanine-2-C^{14} into the NA of 6-Mercaptopurine resistant and thioguanine dependent L1210 leukemic cells is to be made in collaboration with Dr. Skipper. Previous evidence showed a most striking difference in incorporation of 8-Azaguanine into sensitive and 8-Azaguanine dependent cells.

3. A study of the incorporation of labelled aminopterin and labelled CF is to be made in collaboration with Dr. Skipper.

4. Work is now underway by Dr. S. E. Reaume, in our Laboratory, aimed at obtaining mutant strains of Escherichia coli and other bacteria which require specific intermediates in the metabolism of folic acid. Attempts are also being made to obtain mutant bacterial strains which are dependent for growth on the presence of antifolic compounds. It is anticipated that analysis of the folic acid metabolism of such organisms will supply information relevant to the metabolism of analogous drug-dependent leukemias.

5. Other studies to be undertaken include an analysis of the folic acid metabolism of the drug-dependent leukemias and a comparison of their metabolic patterns with those of drug-sensitive and drug-resistant leukemias. Specifically, it is of interest to determine whether anti-folic dependent leukemias metabolize folic acid by means of the same intermediate compounds as do the sensitive lines, or whether the advent of dependence has been accompanied by the elaboration of an alternate pathway in which the antimetabolite plays a role. Another alternative to be explored is that these drug-dependent leukemic lines differ quantitatively, rather than qualitatively, in their folic acid metabolism, as compared to the normally sensitive lines. The work to be carried out in this Laboratory will be aimed at discriminating between these and other alternatives by means of quantitative studies of the folic acid metabolism of the anti-folic-dependent leukemic tissue in vitro. At the present time, chromatographic and spectrophotometric methods are available for the separation and identification of folic acid metabolites. It is anticipated that the use of mutant bacterial strains mentioned above will also facilitate identification of metabolic intermediates.

6. Experiments are now in progress exploring the possibility of inducing drug resistance or dependence in drug-sensitive leukemic cells by means of cell-free extracts of the variant forms. The objective is to determine whether leukemic cells produce "transforming" substances which may play a part in the subsequent development of resistance to

antifolic and antipurine drugs. Subsequent work along these lines would be aimed at elucidating the extent to which such "transformation would occur under normal conditions *in vivo*.

7. Recent investigations by Dr. Shacter have disclosed that one of the antifolic agents, aminopterin, when administered to rats led to decreases in the diphosphopyridine nucleotide (DPN) levels of the liver. *In vitro* incubation of liver homogenates with aminopterin resulted in lowered respiration and DPN content. Adenosinetriphosphate (ATP) specifically counteracted the inhibitory action. Since DPN in the organism is synthesized from ATP and nicotinamide mononucleotide, the results suggest that aminopterin may prevent DPN formation by interfering with the synthesis of the precursor, ATP.

In a study on the influence of dinitrophenol on respiratory and phosphorus interrelationships of Ehrlich ascites tumor cells, a relationship between the respiratory rate, formation of labile phosphorus (ATP) and mitotic activity was demonstrated. In view of these actions of aminopterin, a similar study seems indicated for the effect of antifolic agents on leukemic cells. To that end, leukemias sensitive and resistant to the action of antifolic agents are being developed in ascites form, and the interrelationships between therespiratory, phosphorylative, and mitotic activities of sensitive andresistant leukemic cells as influenced by antifolic, and perhaps other agents, will be determined.

The methods and procedures will, for the most part, be similar to those used in the studies on Ehrlich ascites tumor cells, with respiration being measured by standard Warburg techniques and uptake of P^{32} being utilized as a measure of cellular phosphorylations.

8. A new transplantable lymphocytic neoplasm, L5178 in DBA/2 mice is of interest because of its extreme sensitivity to all antipurine compounds tested to date. It is proposed to initiate work of this particular line aimed at studying synergism between the antifolic and antipurine compounds. In collaboration with Dr. Arnold Welch of Yale University studies are now underway in the effects of 6-Azathymine and of certain derivatives, especially the desoxyriboside, on inhibition of leukemic cell growth. It has been found by Welch *et al.* that in *S. faecalis* that this analog of thymine was particularly effective at a critical growth period in the presence of antifolics.

PROJECT REPORT FORM (Cont'd)

0. CY-427
SERIAL NO.

1. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$92,812	6	7	13	6	7	13	
FY 1956	$112,787	7	9	16	7	9	16	

2. BUDGET ACTIVITY:

RESEARCH ☆ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

3. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Laboratory of Pathology, NCI.
Laboratoryof Biophysics, NCI.
Laboratory of Infectious Diseases, NMI.
Southern Research Institute, Birmingham, Alabama.
Department of Pharmacology, Yale University Medical School.

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

15. NC1-427
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Law, L. W.: Recent advances in experimental leukemia. Cancer Res. 14: 695-709, 1954.

Lorenz, E., Law, L. W. and Congdon, C. C.: The role of bone marrow and spleen in induced and spontaneous lymphatic leukemia. Ciba Foundation Symposium on Leukaemia Research. J. & A. Churchill Ltd. London 1954. pp. 189-195.

Law, L.W.: The phenomena of resistance and dependence in leukaemic cells. Ciba Foundation Symposium on Leukaemia Research. J. & A. Churchill Ltd. London 1954. pp. 105-129.

Williams, A. D., P. J. Winzler and L. W. Law: The effects of A-methopterin on the in vitro incorporation of $P^{32}O_4$ into normal and leukemic mouse tissue. Cancer Res. 14: 135-138, 1954.

Law, L.W.: Maternal influence on experimental leukemias of mice. Ann. N. Y. Acad. Sci. 57: 575-583, 1954.

Law, L. W.: Genetic studies in experimental cancer. Academic Press, N. Y. Vol. II. pp 281-352, 1954.

Law, L. W.: Studies in transformations in leukemic cells of the mouse. J. Nat. Cancer Inst. 15: 817-836, 1954.

Law, L. W., Taormina, V. and Boyle, P. J.: Response of acute lymphocytic leukemias to the purine antagonist, 6-mercaptopurine. Ann. N. Y. Acad. Sci. 60: 000-000, 1954.

Mandel, H. G. and L. W. Law: The effect of 4-amino-5-imidazolecarbox amide on the carcinostatic action of 8-Azaguanine. Cancer Research (In press)

(Cont.)

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

Stewart, Sarah E.: Homotransplants in mice. J. Nat. Cancer Inst. 14: 1187-1193, 1954.

Stewart, Sarah E.: Neoplasms in mice inoculated with cell-free extracts or filtrates of leukemic mouse tissues. I. Neoplasms of the parotid and adrenal glands. J. Nat. Cancer Inst. (In press).

Stewart, Sarah E.: Neoplasms in mice inoculated with cell-free extracts or filtrates of leukemic mouse tissues. II. Leukemia in hybrid mice produced by cell-free filtrates. J. Nat. Cancer Inst. (In press).

Stewart, Sarah E.: Adrenal gland neoplasms in mice resulting from the inoculation of extracts prepared from Strain C3H mouse leukemic tissues. Science (In press).

Study of chemically defined medium and cell nutrition.

(NCI-428, a thru h)

PROJECT REPORT FORM

1. National Cancer Institute
2. Biology Laboratory or Branch
3. Tissue Culture Section or Service
4. Location (if other than Bethesda)
5. ICI-428(e) Serial No

The effect of serum fractions on proliferation of strain L cells in vitro.
Project Title

Sanford, K.
Principal Investigator(s)

Bryant, J., McQuilkin, W., Fioramonti, M., Westfall, B.B., Evans, V.J. and Earle, W.R.
Other Investigators

PROJECT DESCRIPTION : Objective - Since washed serum residue after ultrafiltration has been shown to be an important component for cell proliferation the objective of this study is to identify the active component or compenents of this residue.

Methods employed: Fractions of serum prepared in this laboratory or prepared commercially are tested on replicate cultures. The effects of these fractions are determined by nuclei counts of the cultures.

Major findings: It has been found that all bovine serum fractions obtained commercially appear to be toxic to the cultures. Gross globulin prepared from horse serum gives almost as good proliferation with certain supplements as whole horse serum. The alpha and beta globulins obtained from the gross globulin after removal of gamma globulin which appears to be relatively inactive give good proliferation of cells. Albumin fraction appears relatively inactive as well as fractions of serum lipids and serum ash.

Significance to cancer research: This is part of the program for obtaining a reproducible, adequate medium made up of defined known constituents. A defined medium is regarded as one of the initial steps toward getting cont rol of external conditions during a study of the transformation of normal to malignant cells.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

and qualitatively
/indistinguishable from those grown in our best naturally occurring medium of horse serum and chick embryo extract.

The results on the defined medium number 2 (without globulin) indicate from the condition of cultures that this medium is as good as or better than embryo extract ultrafiltrate. This finding appears to indicate that the effective components in the embryo extract ultrafiltrate have already been replaced by chemically defined materials, and a simplification of the fraction already chemically defined. Therefore, the problem resolves itself into a further study of the constitution and action of gross globulin fraction of the horse serum.

Significance: A chemically defined medium is of primary significance in order to determine differences that may exist in function and chemical behavior of malignant cells in vitro, as compared with the normal cell from which they arise. Such a medium might be useful in making tissue cultures an adequate tool for chemotherapeutic and virological studies as well as research in the nature and origin of the malignant transformation.

Proposed course of project: The effect of the addition and deletion of other small molecule components to these media will be immediately examined. Further fractionation of the gross globulins will be attempted and the effect of the addition of these fractions will be determined.

PROJECT REPORT FORM (Cont'd)

15. NC1-428(a)
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Studies of certain defined media for testing proliferation in replicate tissue cultures. V.J.Evans, J.C. Bryant, K.K. Sanford, and W.R.Earle. Annals of New York Academy of Sciences 58(7): 971-1326, 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM (Cont'd)

10. NCI-428 (a thru h)
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 71,748	10	7	17	6.0	3.0	9.0	
FY 1956	$ 71,604	10	7	17	6.0	3.0	9.0	

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM

1. National Cancer Institute
2. Biology
LABORATORY OR BRANCH

3. Tissue Culture
SECTION OR SERVICE
4. LOCATION (IF OTHER THAN BETHESDA)
5. ZC1-428(2)
SERIAL NO

Nutrition of Mouse Liver Epithelium.
PROJECT TITLE

Westfall, B. B. and Evans, V.J. and Earle, W.R.
PRINCIPAL INVESTIGATOR(S)

Hawkins, N.M., Peppers, E.V. [redacted].
OTHER INVESTIGATORS

PROJECT DESCRIPTION: A) Development of studies on the nutritional requirements of C3H mouse liver cells. B) Metabolism studies of the liver cells in a defined medium. C) Enzymatic studies of liver cells in vitro.

Objective: To design and test a defined or semi-defined medium which will be sufficiently reproducible to allow determination of effects of individual components of the medium. To determine immediately the metabolic fate of the amino acids. To determine the requirements and activity of the vitamins. To compare similarities and differences of two cell lines from the same [redacted].

Methods employed- By use of replicate tissue cultures and the count enumeration method determine the effects of the medium devised on proliferation of the liver cells. Similarly determine the fate of the amino acids, by incorporation of radioisotopes of tagged amino acids. By studies of nutritional requirements and enzymatic studies determine the utilization of amino acids, purine bases, pyrimidines, carbohydrates and salts.

Major findings: To date exploratory experiments have been run with a medium containing amino acids, vitamins, purines, pyrimidines, dextrose and twice crystallized ovalbumin. The medium is adequate for short-term cultivation of the cells. The medium may serve as a reference medium for future modifications.

Significance: The significance of this study will be to learn if mammalian cell metabolism in vitro is different from bacterial

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

metabolism and whether or not single cell metabolism will be different from the whole hosts - with a base line of on normal liver information, attempts to ascertain the difference between the normal and malignant liver cell will be made.

Proposed course of project: To continue the research as defined above and by the methods suggested above.

PROJECT REPORT FORM (Cont'd)

10. NCI-428
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955								
FY 1956								

12. BUDGET ACTIVITY:

RESEARCH ☐ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Steinman, H.G.)- NMI, LCI
Phillips, L.)

Collaborative Inter-Institute study, for more detail see report of Dr. H.G.Steinman, NMI, LCI

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM

1. National Cancer Institute
2. Biology
LABORATORY OR BRANCH

3. Tissue Culture
SECTION OR SERVICE
4. LOCATION (IF OTHER THAN BETHESDA)
5. NCI-428(8)
SERIAL NO

A study of the alpha-keto acids in the medium used for the cultivation of cells.
PROJECT TITLE

Westfall, B.B. and Peppers, E.V.
PRINCIPAL INVESTIGATOR(S)

Bryant, J.C., Schilling, E.L. and Earle, W.R.
OTHER INVESTIGATORS

PROJECT DESCRIPTION,: Objectives - Anoxia has been reported to increase the concentration of pyruvic acid in the blood of the intact animal. Further, anaerobic conditions during growth have been reported to favor the production of malignancy in vitro. It was deemed of interest to see if there was any change in pyruvic and other alpha keto acids in the medium during growth of the cells under conditions of continuous gas exchange on shaker flasks.

Methods employed: The total alpha-keto acids were converted to dinitrophenylhydrazones and separated from other materials by solvent partitioning, They were then separated from each other by paper chromatography.

Major findings: To date only the strain HeLa has been examined under these conditions. This strain showed a slow increase in the total keto acids up to approximately three times normal for mouse serum in 28 days.

Significance to cancer research: At present it is not known whether the slow rise in keto acid concentration is a reflection of a peculiar type of metabolism of the strain HeLa cells, an aspect of the growth of cells under partial anaerobiosis or blocking of transamination of certain amino acids under these conditions, perhaps by exhaustion of the key amino acid content. Since any one of these may be of importance to the problem of malignance further study with other cell strains and greater oxygenation is contemplated.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM

1. National Cancer Institute — 2. Biology Laboratory or Branch

3. Tissue Culture Section or Service — 4. Location (if other than Bethesda) — 5. ZC1-428(4) Serial No.

A study of the amino acid content of medium before and after growth of cells in it.
Project Title

Westfall, B. B. and Peppers, E.V.
Principal Investigator(s)

Bryant, J.C., Schilling, E.L., and Earle, W.R.
Other Investigators

PROJECT DESCRIPTION : Objective -Study of the free amino acid content of the standard medium supplied to certain cell strains in the laboratory had revealed relatively low free amino acid content. A study by the Winnicks had shown that there was very rapid uptake of certain free amino acids from similar medium. It therefore appeared to be desirable to see if certain of the amino acids were rapidly depleted to the stage where their concentration would be limiting factors in growth.

Methods employed: The cells were grown in shaker flasks with continuous gas exchange (Bryant, Schilling, Earle) and the fresh and used medium removed at intervals for analysis. Total amino acids were estimated by the ninhydrin colorimetric procedure and individual ones after separation on paper or by microprocedures such as conversion of serine to formaldehyde, alanine to acetaldehyde etc.

Major findings: The study is continuing. However, to date it has been ascertained that certain of the amino acids present in low concentration in the medium supplied are apparently exhausted in 48 hours exposure to the cells under conditions where growth is rapid and the inoculum high.

Significance to cancer research: It has been reported that anaerobiosis (Cameron, Glodblatt) is a factor in producing malignancy in cultivation of cells in vitro. Part of the reason for developing continuous gas exchange systems for the cultivation of cells is to overcome any tendency to anaerobic conditions. No data exist on the metabolic behavior of cells under a system such as we are

NC1-428(F)

now setting up. Hence this study is a portion of an extensive projected study on the metabolism of cells under varying conditions.

Proposed course of project: The above study has so far been limited to the human malignant epithelial strain HeLa. It is proposed to carry out a similar study with the strain L if it can be adapted to survival under the shaker flask conditions and with the strain 721 liver epithelial cells if they likewise can be grown under the continuous shaking-continuous gas exchange conditions. These conditions make available relatively large amounts of fluid (supernatant) for analysis.

5. NCI-428(F)
SERIAL NO.

6. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

The amino acid content of the ultrafiltrate from horse serum. Westfall, B.B., Peppers, E.V., Sanford, K.K., and Earle, W.R., J. Nat. Cancer Inst. 15, 27-35 (1954)

The free and combined amino acids in the protein-free ultrafiltrates from whole chick-embryo extracts. Westfall, B.B., Peppers, E.V., and Earle, W.R. J. Nat.Cancer Inst. 15, 433-438, 1954.

. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

National Cancer	2. Biology		
INSTITUTE	LABORATORY OR BRANCH		
Tissue Culture	4.		5 ACI-428(C)
SECTION OR SERVICE	LOCATION (IF OTHER THAN BETHESDA)		SERIAL NO

Development of a defined medium for the cultivation of strain L cells in vitro.
PROJECT TITLE

Evans, V. J.
PRINCIPAL INVESTIGATOR(S)

Bryant, J.C., McQuilkin, W.T., Fioramonti, M.C., Westfall, B.B., Earle, W.R. and Sanford, K.K.
OTHER INVESTIGATORS

PROJECT DESCRIPTION: Objective - To develop a satisfactory defined medium adequate for producing proliferation of a selected strain of animal tissue cells to study its metabolic behavior.

Methods employed: Replicate cultures are prepared and enumerations of their nuclei are made after exposure for varying intervals to the defined media. Such media are devised around the collected information on the nutrition of bacteria, mammalian and avian cells in vitro and the data obtained to date from the physical and chemical fractionation of the naturally occurring medium on which the strain L cells are maintained.

Major Findings: - Several media have been devised and tested. The first contained amino acids and amides, vitamins, purine bases and pyrimidines, dextrose, ribose, inorganic salts, ovalbumin and methocel. From this medium minor proliferation was obtained. The second medium is a decided improvement over the first. The third medium causes rapid cell proliferation. A marked difference in the effect in the morphology of the cells appears in the cultures grown on the second medium from that seen in cultures grown on the third medium. Amino acids and amides, vitamins, coenzymes, methyl esters of unsaturated fatty acids, cysteine and glutathione, cholesterol, nucleic acid derivatives, carbohydrate sources and inorganic salts are used in both the second and third medium. No antibiotics or ~~anti~~fungicides are included in any of these media. The third medium includes in addition a small amount of gross globulin fraction isolated from horse serum. The addition of this fraction from globulin gives a medium in which the cultures appear both quantitatively

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

NC1-428(2)

Proposed course of project: An additional experiment is being carried out to complete this stage of the project.
(See project on fractionation of serum.)

PROJECT REPORT FORM

1. National Cancer Institute — 2. Biology — LABORATORY OR BRANCH

3. Tissue Culture — SECTION OR SERVICE — 4. — LOCATION (IF OTHER THAN BETHESDA) — 5. ZC1-428(L) SERIAL NO

Fractionation of serum proteins for supplementation of defined mediums.

PROJECT TITLE

B.B. Westfall

PRINCIPAL INVESTIGATOR(S)

Peppers, E.V., Sanford, K.K., Evans, V.J., and Earle, W.R.

OTHER INVESTIGATORS

PROJECT DESCRIPTION: Objective - Serum, freed of diffusible materials, has been shown to have important growth enhancing properties when used with certain defined substances. A study has been undertaken to see if activity could be attributed to a single fraction or whether the whole serum was irreplaceable.

Methods employed: The major fractionation has been carried out on previously frozen serum by the Cohn method 10 for ACD plasma. The usual Cohn fractions obtained commercially are toxic to the cells. Hence this has necessitated extensive changes in the preparation. Paper electrophoresis and turbidity-precipitation methods have been used to examine fractions obtained.

Major findings: All fractions exerted some stimulating effect (Sanford, et al.), with the globulins being more favorable. This may, however, reflect partly difficulty in obtaining a completely non-toxic albumin fraction. None of the fractions were completely pure. The gross globulin fraction has 3 per cent albumin with it and the gross albumin had 2 per cent alpha globulin and a small quantity of acidic muco-protein.

Significance to cancer research: This is part of the program for obtaining a reproducible, adequate medium made up of defined known constituents. A defined medium is regarded as one of the initial steps toward getting control of external conditions during a study of the transformation of normal to malignant cells.

Proposed course of project: Sanford, et al. have very nearly completed the first stage on the study. More needs to be done on conditions of

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

NCI-428(d)

fractionation to eliminate toxicity and increase purity of the fractions. Also more work is needed as to the quantity of the serum fraction used. We have assumed that in the screening test the protein should be at approximately the level represented by the serum when used in the standard medium for cultivation of certain cells. Obviously many of these fractions are present in plasma at much lower concentrations than this.

PROJECT REPORT FORM

1. National Cancer Institute 2. Biology
INSTITUTE LABORATORY OR BRANCH

3. Tissue Culture Section 4. 5. (C1-428(C))
SECTION OR SERVICE LOCATION (IF OTHER THAN BETHESDA) SERIAL NO

Study of the effect of fractions of whole egg ultrafiltrate on cell proliferation.
PROJECT TITLE

6. Sanford, K.K., Westfall, B.B. and Earle, W.R.
PRINCIPAL INVESTIGATOR(S)

7. McQuilkin, W.T., Fioramonti, M.C., Bryant, J.C. amd Evans, V.J.
OTHER INVESTIGATORS

PROJECT DESCRIPTION : The objective of this project is the separation of the whole egg ultrafiltrate into various fractions for testing on growth of cells in an effort to localize the active components for cell proliferation.

Method: Ether extracted and ether-alcohol extracted supernatants and residues of lyophilized egg ultrafiltrate are being tested in combination with washed horse serum proteins, amino acids, vitamins, and ATP to determine the effects of these various fractions on growth of strain L cells.

Major findings: The results of the first experiment suggest a partition of active components between the supernatant and residue, obtained from a 70 percent alcohol extraction of the ether residue.

Significance to Cancer Research: The identification of the active components of the whole egg ultrafiltrate for cell proliferation may possibly be of importance in the development of a practical chemically defined proliferative medium.

Proposed course of project: Experiments will be continued on a secondary scale to localize the active component.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM

1. National Cancer Institute
2. Biology
LABORATORY OR BRANCH

3. Tissue Culture
SECTION OR SERVICE
4.
LOCATION (IF OTHER THAN BETHESDA)
5. 101-4ZF(R)
SERIAL NO

Development of whole egg extract ultrafiltrate as a substitute for 9 day chick embryo extract ultrafiltrate for the proliferation of cells.
PROJECT TITLE

Evans, V.J., Earle, W. R. and Westfall, B.B.
PRINCIPAL INVESTIGATOR(S)

Bryant, J.C., McQuilkin, W.T., Fioramonti, M.C., Sanford, K.K.
OTHER INVESTIGATORS

PROJECT DESCRIPTION : Objective - To prepare egg ultrafiltrate and to test the proliferative ability of fertile and non-fertile whole egg unincubated egg extract ultrafiltrate with quantitative and qualitative methods.

Methods employed - Replicate cultures of strain L cells were prepared and cultured on the egg extract ultrafiltrates. Enumeration of nuclei of cultures after varying intervals of exposure were used to determine the effect of the egg ultrafiltrate. Long term experiments over several months were also made.

Major findings - Quantitative experiments show that 0 day egg extract ultrafiltrate at a 20% by volume level was equivalent to 20% by volume of chick embryo extract. Studies on cultures maintained on this egg ultrafiltrate for over 4 months show this extract ultrafiltrate would substitute for 9 day chick embryo extract for other strains of cells besides strain L, namely a mouse liver epithelium, and a strain of cells isolated from a human cervical carcinoma (HeLa).

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Significance: Per egg unit, larger volumes of active proliferation promoting material is available from the whole egg than from saline extraction of a 9-day chick embryo. Such large volumes are in great demand for 2 of the more recent advances in tissue cultures. The material serves as an economical substitute for chick embryo extract in the rapidly agitated massive cultures. For significance of this material to cancer research see report on cultivation of massive cultures (Earle, Schilling and Bryant). Since this medium can be used on the large culture, the studies on the metabolism of these cultures are rapidly accumulating (Westfall, Earle, Bryant and Schilling) with the expectation that the data will point up differences between highly malignant cells or cells from malignant tissue and tissue cultures arising from normal tissue. The material, since it has proved to be equivalent to 9 day chick embryo extract, and is also available in comparatively large volumes serves as sources of proliferation- producing material for analytical studies of the individual factors responsible for this proliferation as well as for a less expensive medium than embryo extract for massive cultures.

Proposed course of project - The immediate plan is to determine a more efficient means of obtaining this egg extract ultrafiltrate for the need specified. Limited experiments will be required to determine if the more economical method of obtaining this material yields a product equally as satisfactory as that from ultrafiltration through collodion membrane.

PROJECT REPORT FORM (Cont'd)

15. NCI-4258(k)
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Studies of Nutrient Media for Proliferation of Mammalian Cells in Vitro. (V.J. Evans. Lecture before the Tissue Culture Association Course at Cooper Town, N.Y. July 27,28, 1954.)

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

Studies of the influence of cell population on proliferation

NCI-429 (a & b)

PROJECT REPORT FORM

1. National Cancer Institute
2. Biology
LABORATORY OR BRANCH
3. Tissue Culture
SECTION OR SERVICE
4.
LOCATION (IF OTHER THAN BETHESDA)
5. NCI-429(d)
SERIAL NO

Growth of massive fluid suspension cultures of animal tissue cells.
PROJECT TITLE

W.R.Earle, Edward L.Schilling, Jay C. Bryant and Virginia J. Evans.
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION Growth of massive fluid suspension tissue cultures of both normal and malignant animal tissue cells. Application of these methods in defining factors responsible for proliferation of representative tissue cell types in such cultures.

Objective : The general objective of this project is to develop methods and equipment for growing massive fluid suspension cultures of mammalian tissue cells. Attainment of this objective during the past year has involved the study of various rates of agitation of the cell suspension, various types of control of air and fluid media and study of other factors which appear to control cell growth under these conditions.

Methods Employed: Much of the work has been done with a standard Brunswick type platform shaker enclosed in a well-insulated incubator box, and operating at various speeds between 6000 and 13,000 revolutions per hour. Special drive mechanism was interposed between the motor and the platform to allow a wide range of shaking speed. A sensitive air-flow control mechanism was devised for leading small uniform increments of humidified air or other gas through each shaker culture flask. Culture flasks were one-liter and 1½ liter flat-bottomed boiling flasks specially redesigned for aseptic gas flow. Appropriate methods were devised for renewing the fluid periodically without appreciable loss of cells. The rate of proliferation of cells in suspension has been determined by the enumeration of cell nuclei.

Major Findings: The major findings of this project during the past year have confirmed previously reported findings that the major

factors which must be controlled in this type of culture growth are: A) Adequate satisfactory nutrient. B) Control of pH. C) Critical control of the cell population density. This differs with various cells. D) Control of the rate and manner of agitation. These variables appear to be identical with those encountered in the commercial growth of fungi in the antibiotics industries. It appears that as a commercially practical nutrient becomes available growth of animal tissue cells in fluid suspension culture may be extrapolated to almost any desired scale. The addition of methycellulose as reported last year has been eliminated as unnecessary.

By this method of culture luxuriant cultures of three quite different strains of cells have been obtained. These were the clone L-929 strain of mouse fibroblasts, the clone 1469 strain of mouse liver epithelium used in this laboratory, and the strain of human cervical carcinoma known as the HeLa strain and now widely used in virological study of polio. Use of this last strain constitutes a particularly significant test of the method since this cell type has been difficult or impossible for many workers to grow at all while it has been generally claimed it would not grow at all in fluid suspension culture but must attach to a solid substrate.

Concepts and methods developed in this shaker-type culture during the last year have already allowed the HeLa cell for instance to be grown in this shaker type culture in lots of about 7 billion cells per flask (probably about 12 grams wet weight). Study of the method indicates an efficiency of utilization of the culture media which is extraordinarily high.

The cultures have adapted themselves extraordinarily well to the study of the influence of the cells on the nutrient. For instance, from a study of the low accumulation of alpha keto acids, together with high dextrose utilization of the HeLa cells, it already appears that these shaker type cultures are probably under far more "normal" conditions than any type culture so far in use in our laboratory.

Some difficulty has so far been encountered in rapidly adapting such coherent cells as the skin epithelium or the fibroblast to growth in these shaker cultures. This problem is under continued study to determine the cause of this difficulty.

Significance to Cancer Research: The results obtained are of major significance in making practical various metabolic studies with normal and with malignant cells in massive cultures. Large uniform cell suspensions in one culture derived from a pure cell strain have not hitherto been available for the extensive type of multiple chemical analyses which would be desirable. Such cultures can now be grown on a large scale. One recent point theoretically developed but not yet published is that it appears possible to mathematically correlate the maximum practical size of a unicentric tissue culture with its QO_2, the intermitotic interval of its cells and their migration rate.

Proposed Course of the Research: This study is being continued as a major project in further developing methods and concepts of the agitated fluid culture, and in making more general application of this type culture in the growth of cells for cancer research and related lines, particularly with respect to cell chemistry and metabolism.

PROJECT REPORT FORM (Cont'd) NCI-429(a)

15. NCI-429(a)
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Influence of Tube Rotation Velocity on Proliferation of Strain L Cells in Surface Substrate Roller Tube Cultures. W. R. Earle, E.L. Schilling, and J.C. Bryant, J. Nat. Cancer Inst. 14(4): 853-864, 1954.

The Growth of Pure Strain L Cells in Fluid Suspension Cultures. W.R.Earle, E.L.Schilling, ~~and~~ J. C.Bryant, and V. J. Evans J.Nat. Cancer Inst. 14(5): 1159-1171, 1954.

Long-Term, Large-Scale Tissue Culture. W.R.Earle. Ciba Foundation Symposium on Preservation and Transplantation of Normal Tissues, London, 1953. pp. 44-59, J.& A. Churchill, Ltd. 1954.

Certain Factors Limiting the Size of the Tissue Culture and the Development of Massive Cultures. W.R.Earle, J.C. Bryant, and E.L. Schilling. Annals of New York Academy of Sciences 58 (7): 1000-1011, 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

NCI-429(a)

PROJECT REPORT FORM

1. National Cancer INSTITUTE
2. Biology LABORATORY OR BRANCH
3. Tissue Culture SECTION OR SERVICE
4. LOCATION (IF OTHER THAN BETHESDA)
5. NCI-429(R) SERIAL NO

Single cell studies and the development of cell clones.
PROJECT TITLE

Sanford, K.K. and Hobbs, G.L.
PRINCIPAL INVESTIGATOR(S)

Earle, W. R.
OTHER INVESTIGATORS

PROJECT DESCRIPTION: The objective of this project is the development of clones of human fascia, human skin, human liver, mouse mammary carcinoma, mouse liver, and four strains of mouse fibroblasts. Each of these clones is desired for specific research projects, which will be considered under other headings. The development of a clone of mouse mammary carcinoma is for use in a collaborative study with Dr.H.B.Andervont on the relation of the milk factor to the malignant cell in tissue culture.

Methods employed: A sieved cell suspension is prepared and drawn into capillary pipettes. Capillary segments of 5 mm. length are cut, each segment containing one isolated cell. The segments are embedded in plasma clot in a Carrel flask, and the culture fluid is renewed thrice weekly.

Major findings: Two clones of cells have already been established, one of human fascia and one of human epidermis. Both of these clones, however, after 7 and 9 months respectively of cultivation in vitro, failed to grow further, possibly as a result of poor lots of human serum used in the culture medium. After 24 unsuccessful attempts to clone the other strains of cells, a modified and improved procedure has just been developed and will now be tested.

Significance to Cancer Research: The development of clones of cells is of fundamental importance for future studies in carcinogenic cell transformations among populations of cells in vitro, virus studies, and in studies of the differences in the physiology and nutrition of malignant cells and the normal cells from which they arise in culture.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Proposed course of project: With this recent improvement in technique, renewed efforts will be made to develop clones of the several human and mouse cell strains listed above.

10. ee1-429 (a+b)
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$ 26,549	6	7	13	3 9	1.5	3 1/3	
FY 1956	$ 26,527	6	7	13	3 9	1.5	3 1/3	

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Studies of human tissue particularly epithelium in culture.

NCI-430 (a thru f)

PROJECT REPORT FORM

National Cancer
INSTITUTE

2. Biology
LABORATORY OR BRANCH

Tissue Culture
SECTION OR SERVICE

4.
LOCATION (IF OTHER THAN BETHESDA)

5. NCI-430(a)
SERIAL NO

Studies on the cultivation of human endocrine secreting tissue in vitro.
PROJECT TITLE

Evans, V.J. and Earle, W.R.
PRINCIPAL INVESTIGATOR(S)

Hawkins, N.M.
OTHER INVESTIGATORS

PROJECT DESCRIPTION : Objective - To cultivate cells of endocrine tissue as made available by the Endocrinology Branch of the National Cancer Institute in order to learn the extent and duration of hormone secretion of isolated cells in vitro.

Methods employed - A human islet tumor of the pancreas, pancreatic tissue from a clinically diagnosed case of islet tumor have been or are being grown in culture for assay of hormone production.

Major findings: The pancreatic tumor tissue established last year showed some insulin by the hormone assay method described in Emmons: Assay Methods. However, this activity was no longer obtained after a short interval of cultivation in measurable amounts in the used fluids from the cultures or in homogenized cells. Presently this strain of cells is being carried to attain if possible large cultures for assays of greater amounts of tissue. A new strain of pancreatic tissue has just been established from tissue obtained from a patient in the Clinical Center. No assays have as yet been made on this tissue.

Significance to cancer research: The development of techniques for growing human endocrine tissues in vitro so that they continue to function may have considerable practical value for therapy. From the point of view of cancer research such endocrine assays under different in vitro experimental conditions serve as valuable indicies of the changes in cells under experimental conditions.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Proposed course of this project: Experiments will be continued to culture various tissues of endocrine origin as they become available from the Clinical Center to determine the duration and amount of hormone secretion and to study the morphology of tissue growth in order to correlate morphology, function and therapy.

Attempts to devise new methods of assay of function by grafting will be taken under consideration if exploratory experiments appear to justify this approach.

PROJECT REPORT FORM (Cont'd)

10. AC1-430(a)
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955								
FY 1956								

12. BUDGET ACTIVITY:

RESEARCH ☐ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Hertz, R., Tullner, W. and Hallman, L. , Endocrinology Branch, National Cancer Institute.

~~None~~

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM

1. National Cancer Institute — 2. Biology — LABORATORY OR BRANCH

3. Tissue Culture — SECTION OR SERVICE — 4. — LOCATION (IF OTHER THAN BETHESDA) — 5. ZC-430 (Ld) — SERIAL NO

Development of strains of human cells in tissue culture for propagation of APC virus.
PROJECT TITLE

Evans, V.J.
PRINCIPAL INVESTIGATOR(S)

Hawkins, W.W.
OTHER INVESTIGATORS

PROJECT DESCRIPTION Objective: To test whether APC respiratory virus (4 types) will propagate in cells in vitro.

Method - Cultures of human liver and human embryo skin grown in vitro for relatively prolonged periods were examined for a cytopathogenic effect and production of complement-fixing antigens. Cultures of human liver, strain 1815, were received. The cultures were washed several times with medium, and were left in 1.0 to 1.5 ml. of the same medium, which consisted of chicken serum 5%, chick embryo extract ultrafiltrate 5%, and Hanks' solution 90%. For experiments with Type 4 APC virus the medium contained Parker's Mixture 199 in place of the Hanks' solution. Cultures were infected with Types 1,2,3, and 4 APC virus, and were observed for cytopathogenic effects. When the effects were marked, the supernatant fluid was passed to a fresh set of cultures in a dilution of 1:10. All four virus types were carried for three serial passages, with production of cytopathogenic changes and production of complement fixing antigen in all sets of cultures. Since culture fluids were changed three times weekly, and passages were made at about ten day intervals there is no doubt that the virus recovered represents newly formed virus and is not residual from the inoculum.

Cultures of human embryo skin were observed for the production of of cytopathogenic changes after the inoculation of Types 1,2, and 3, and characteristic effects were seen with each virus. No attempt was made to carry the viruses in serial passage in this tissue.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Significances of Findings: In order to determine whether it is reasonable to assume that any human cell line will propagate this virus several strains of cells were tested. Should this assumption be proved to be correct - and to date all human lines tested will propagate it - the need for large scale production becomes most pertinent for the further study of the A.P.C. virus group and for the production of virus for vaccine production from a strain of cells not obtained from cancer tissue.

Proposed course of project : De velopment of strains of human cells are desired in large masses, capable of propagating A.P.C. virus which has considerable significance in bringing about loss of man hours in both civilian and military population. Continued studies must be made to develop strains of cells which will grow on a semi-defined medium or on a media low in non-viral protein such as horse serum for human cells. Such strains must be tested for viral propagation and non-malignancy. This presupposes continued work in methods to develop large cultures such as shaker cultures and to determine whether human cells in culture are malignant. Transplantation of tissue into the interior of the mouse eye, x-irradiation and cortisone treatment of animals will be given consideration. Methods of controlling possible development of malignancy by cells in tissue culture must be devised and tested for their validity.

PROJECT REPORT FORM (Cont'd)

0. ac1-430(h)
SERIAL NO.

1. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955								
FY 1956								

2. BUDGET ACTIVITY:

RESEARCH ☐ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

3. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Collaborative Inter-institute study, for more detail see report of Dr. Robert J. Huebner, LID, NIH.

None

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

STANDARD FORM NO. 64

NCI-430(b)

Office Memorandum • UNITED STATES GOVERNMENT

TO : Dr. Nider, thru Dr. Andervont DATE: Jan. 6, 1955

FROM : Dr. Earle

SUBJECT: Progress reports submitted by the Tissue Bank of the U.S.Naval Hospital.

The project report submitted on the collaboration studies with the U.S. Naval Hospital Tissue Bank represent work done in some part with the facilities of the Tissue Culture Section, Laboratory of Biology, NCI. A good part of the work, however, has been done by the Navy Tissue Bank in their own facilities under a system of consultative collaboration.

PROJECT REPORT FORM

National Cancer Institute	2. Biology Laboratory or Branch		
Tissue Culture Section or Service	4.	Location (if other than Bethesda)	ZCI-430(C) Serial No

Project #1

A Study of human epithelial cultures.
PROJECT TITLE

Evans, V.J. and Earle, W.R.
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION: Objective- a) To produce and maintain as many series of human epidermis cultures as are necessary for the understanding of its behavior. b) To determine by methods available, the minimum amount of time required to cultivate these cells in quantities sufficient for its clinical application in thermal and tramatic injuries. c) To identify these series of cells as to their primary origin, i.e., connective tissue or epithelial. d) To adapt these series of cells to a media of heterologous composition to assist in the study of the problem of homografting. e) Once these cells are available in sufficient quantities, to determine the most practical, inexpensive and less toxic means of preservation for extended periods of time. f) Animal experimental procedures to follow the behavior of normal cultured cells in vivo for differentiation and malignancy. g) To produce strains of epithelial cells derived from endocrine tissue in order to study their products of metabolism and excretion.

From the objectives stated, collaborative projects have been instituted. These are: objectives C, D and E, and are therefore assigned Projects Numbers 2,3 and 4. (NMC)

Methods: a) Split thickness skin and endocrine tissue procured from the Tissue Bank, Naval Medical School is subjected to varying concentrations of trypsin. The epithelial elements are removed after the digestion of its collagen fibers, suspended in human stock media and planted in Earle T-80 flasks. The success of this procedure is not always certain, and yields positive results in approximately only 50% of the attempts. After varying intervals of time (1 to 3 weeks) migration of the epithelial cells is seen. b) Animal

experimental procedures, approximating those of its clinical application, have been instituted in limited numbers. Normal mouse epithelial elements have been planted under a plastic observation chamber on the back of C3H mice. To date, microscopic evaluation of the post mortum sections have not been completely evaluated. Animals have just been sacrificed after having received under plastic observation chambers: 1) Trypsin split suspension of epidermal cells; 2) Trypsin split epidermal sheets; 3) Trypsin split dermal elements; 4) Trypsin digested full thickness skin; and 5) Normal Neonatal skin.

Major findings The major findings of this project has been restricted to the cultivation in vitro of a strain of normal human epithelium, which has been subcultured through out the calender year.

Significance: There is no report in the literature of a strain of normal human epithelial cells, derived from the epidermis, having been cultivated over this period of time, after trypsin splitting.

Projected plans: a) To continue the study of autogenous cultivated epithelial cells in the experimental animal. b) By correlating information secured from projects Number 2, 3 and 4 be able to produce, maintain and preserve large masses of cultured epithelial cells for clinical application, if practical.

NCI-430(f)

NCI-430(f)
SERIAL NO.

BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955								
FY 1956								

BUDGET ACTIVITY:

RESEARCH ☒ (year) ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Kindred, R. Lt(MC) NNMC NMS Tissue Bank
Hyatt, G. LCDR (MC) NNMC NMS Tissue Bank
DRANEY, J LTJG (MC) NNMC NMS Tissue Bank
PERRY, V. HM1 NNMC NMS Tissue Bank

IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM

1. National Cancer INSTITUTE 2. Biology LABORATORY OR BRANCH

3. Tissue Culture Section SECTION OR SERVICE 4. LOCATION (IF OTHER THAN BETHESDA) 5. NCI-43-(2) SERIAL NO

Project #2

Anterior chamber injections.
PROJECT TITLE

Earle, W.R. and Evans, V.J.
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION: a) To determine the occurence of tumors in the anterior chamber of the eye of C3H mice following injection of various strains of malignant and non-malignant cells, as compared to injections of normal human skin epithelium. b) The redifferentiation pattern of trypsin digested skin epithelium as compared to strains of normal human fibroblasts.

Method: C3H mice were prepared in the usual manner, after receiving 350 R X irradiation. Stab incisions were made medial to the limbus of sufficient size to introduce a .1 mm pipette. A suspension (.1 ml) reduced to 1/10 original diluent were innoculated with the animal returned to its cage. After varying intervals of time (1 to 6 weeks) the animals were sacrificed and the eyes prepared for sectioning.

Major findings: Of the series completed to date, which include five strains of cells, Namely:

1952 human skin (normal)
1769 human skin (normal)
1863 human skin (normal)
1881 human cervical epithelioma
1942 human fascia (normal)

only 1881 has produced a tumor of significance.

Significance: a) From this small series of anterior chamber injections, no significance can be drawn. By continuing the procedure, it is hoped to learn whether or not normal tissues, when cultivated in vitro will or will not produce a tumor in the x-irradiated heterologous host. b) That established strains of cells of epithelioma origin when injected in the X-irradiated heterologous

host, will establish tumors in a large percentage of the cases, morphologically resembling the original tumor. c) No pattern of redifferentiation has been formulated.

Projected plans: a) To continue the present project, utilizing all series of cells carried in this laboratory, with the objective as stated earlier. b) To supplement this small project with an occasional intraperitoneal implant of tissue within an Algire chamber.

PROJECT REPORT FORM (Cont'd)

10. ACJ-430(C)
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955								
FY 1956								

12. BUDGET ACTIVITY:

RESEARCH ☐ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Kindred, Robert, LT(MC) NNMC, NMS Tissue Bank

Hyatt, R. LCDR(MC) NNMC, NMS, Tissue Bank

Perry, V. H. 1, NNMC, NMS, Tissue Bank

Draheim, J. LTJG (MC) NNMC, NMS, Tissue Bank

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM

1. National Cancer Institute — 2. Biology Laboratory or Branch

3. Tissue Culture Section or Service — 4. Location (if other than Bethesda) — 5. ZC1-430(E) Serial No

Project #3 - Adaptation series.
PROJECT TITLE

Earle, W. R. and
Evans, V.J.
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION : To adapt a strain of cells to a media of heterologous composition.

Method: a) Present experimental studies include; the introducing of media of varying concentrations of human and horse serum to a number of well adapted human malignant cells (e.g. HeLa) attached to the floor of Carrel D-3.5 and Earle T-30 flasks and well adapted to human sera. After an interval of 120 hours, the less foreign and consequently less toxic of the serum concentration will permit the subculturing of a substrain of cells for study. The adaptation of a human malignant strain has been completed, while the method of adapting a strain of normal human fibroblasts is in progress, and shows very clear possibilities.

Major findings: By a system of dilution in serum concentration, a series of human malignant tissue (1881) has been adapted to a media of heterologous (horse) media (1985).

Significance: Practically, the existence of a substrain of HeLa (human malignant epithelial cells) that can be cultivated in large masses in other than homologous serum offers extensive opportunity for the use of these cells in human viral studies. The mechanism of adaptation should be an interesting situation for study by the immunologist interested in the problem of homografting.

Projected plans: a) Present experimental studies include the reducing of the concentration of horse serum, yielding perhaps a less prolific substrain of cells (though influenced by less inhibitive sera for viral studies), and b) the adaptation of a

NCI-430(2)

strain of normal human cells to a media of heterologous media.

PROJECT REPORT FORM (Cont'd)

10. AC1-430(e)
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955								
FY 1956								

12. BUDGET ACTIVITY:

RESEARCH ☐ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Kindred, R. Lt.(MC) USNRC, NMS, Tissue Branch
Hyatt, R. LCDR (MC) USNRC, NMS, Tissue Branch
Perry, V. (HM1) USNRC, NMS, Tissue Bank.
DRAHEIM, J LTJG (MC) USNR, NMS, Tissue Bank

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM

1. National Cancer Institute — 2. Biology Laboratory or Branch

3. Tissue Culture Section Section or Service — 4. Location (if other than Bethesda) — 5. NCI-1430(F) Serial No

Project #4.

Preservation of cultured cells.
Project Title

Evans, V.J. and Earle, W. R.
Principal Investigator(s)

Other Investigators

PROJECT DESCRIPTION : Objective - Now that cells can be cultured in large masses, it is desirable to be able to preserve these cells in a latent state, for extended periods of time. The most promising method reported to date, has been reported by Polge, C. (Nature 164:666:1949) who used glycerine impregnation for preserving bull spermatazoa. The Naval Tissue Bank, Naval Medical School has utilized this method for the preservation of two types of tissue; skin and cornea. An evaluation of these two tissues preserved in glycerine therefore should be made. The most significant factor of this method is namely: (1) Length of preservation (1 year and longer); (2) the inexpensive material required (Dry ice and Glycerine); and (3) the least toxic as compared to other methods.

Method Skin preserved by the glycerine method has been used clinically at the USNH Bethesda, with results approximating those of skin preserved in nutrient media at +4°C. Cornea has been preserved in glycerine and stored at dry ice temperatures and used clinically, though not at the U.S.Naval Hospital. By using control cultures of fresh tissues (skin and cornea) it is possible to evaluate the success of the glycerine method of preservation over a given period of time. Skin and cornea will be procured from the Tissue Bank, Naval Medical School, and evaluated after immediate and prolonged terms of storage. The skin to be preserved is wrapped around a glass cylinder and immersed in a 15% concentration of glycerine USP, and 85% Earles saline for 1 hour. The excess of solution is decanted and the cylinder of skin placed in a double jar unit for storage at dry ice temperatures. Cornea are treated in a similar manner but without fixation to glass. A limited series of fresh cornea have been planted in Carrel D-3.5 and Earle T-30

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

flasks and have demonstrated proliferation of epithelium, substantia propria and endothelium.

Major findings: Controls for the glycerine method of preservation have been obtained.

Significance: Corneal endothelial cells (which are required in a viable state in Keratoplasty) may assist in establishing criteria for the preservation of cells cultured in large masses and stored in glycerine. If it can be demonstrated that corneal endothelial cells, which are preserved by glycerine impregnation and stored at dry ice temperatures exhibit viable cells in vitro, possibly, cells of less specific function may be preserved in a like manner.

Projected plans: a) Fresh and glycerine impregnated skin and cornea will be procured from the Tissue Bank, Naval Medical School, for the purpose of evaluating the application of this method to the preservation of cells cultivated in large masses by the shaker technique. b) The innoculation with glycerine of various strains of cells carried in this laboratory for the purpose of: (1) determining the tolerance of isolated cells to glycerine, (2) rate of freezing and thawing with the retention of viability, and (3) the maximum length of preservation at dry ice temperatures.

PROJECT REPORT FORM (Cont'd)

LW-430(d)
SERIAL NO.

BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955								
FY 1956								

BUDGET ACTIVITY:

RESEARCH ☐ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Kindred, R. NNMC NM 5 Tissue Bank
Hyatt, S.Lt. NNMC NMS Tissue Bank
Perry, V. HM 1, NNMC, NMS, Tissue Bank
Draheim, J LTJG (MC) NNMC, NMS, Tissue Bank

IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM (Cont'd)

. UCI-430 (a thru f)
SERIAL NO.

. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$11,482	3	7	10	.6 2/3	1	1.6 2/3	
FY 1956	$11,501	3	7	10	.6 2/3	1	1.6 2/3	

. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Studies of cell transformations *in vitro*.

NCI-431 (a thru c)

PROJECT REPORT FORM

1. National Cancer Institute | 2. Biology Laboratory or Branch

3. Tissue Culture Section or Service | 4. Location (if other than Bethesda) | 5. ZCI-431 (B6) Serial No

A study of the tumor-producing capacity of two substrains of clone 1328.
PROJECT TITLE

Sanford, K.K.
PRINCIPAL INVESTIGATOR(S)

Hobbs, G.L., Fioramonti, M.C. and Earle, W. R.
OTHER INVESTIGATORS

PROJECT DESCRIPTION Objective The objective of this study is to determine why two substrains of clone 1328 differ in their capacity to produce tumors in strain C3H mice. One of these, substrain III, produces 1 percent tumors; the other, substrain VII, produces 97 percent tumors. Both of these substrains arose from the same single cell. Substrain III produces an increased number of tumors if the cells are injected into x-irradiated mice. Tumors of substrain III arising in x-irradiated mice grow on subinoculation in all non-irradiated mice injected. A second objective of this study is to explain this change in tumor-producing capacity of substrain III cells after one passage in an x-irradiated strain C3H mouse.

Methods employed: Strain C3H mice are injected with cells of substrain III or VII, and after a two week period are challenged with an injection of either substrain to determine whether immunity has developed. Cells or tumor tissue are also injected into foreign strains of mice having different histocompatibility alleles to determine whether a genetic change in histocompatibility has occurred. Tumor tissue arising from both substrains is returned to culture, and the behavior of the cells tested by subsequent injection into strain C3H mice. Cells of both substrains are grown in diffusion chambers in the mouse to determine their behavior in the mouse.

Major findings: It has been found that substrain III cells immunize or increase the resistance of the strain C3H mouse to subsequent implantation of cells of substrain III, and also to a limited extent to cells of substrain VII and clone 929. Cultures or tumor tissue of substrain VII will grow in a limited number of strain DBA and A mice, but both substrains of cells seem to have a specificity

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

not found to grow in other strains of mice having different histo-compatibility alleles.

Proposed course of project: The specificity of the immune reactoon induced in the strain C3H mouse is being investigated. A manuscript is in preparation. Since tumor strains differing markedly in non-irradiated animals have been developed from this clone, the homogeneity of the cell population will be tested by means of single cell isolations.

PROJECT REPORT FORM

1. National Cancer Inst. (INSTITUTE) 2. Biology (LABORATORY OR BRANCH)

3. Tissue Culture (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. ACI-43(a) (SERIAL NO)

A study of the tumor-producing capacity of clone 929 of strain L.
PROJECT TITLE

Sanford, K.K.
PRINCIPAL INVESTIGATOR(S)

Hobbs, G.L., Fioramonti, M.C., and Earle, W.R.
OTHER INVESTIGATORS

PROJECT DESCRIPTION Objective: - The objective of this study is to determine why cells of clone 929 of strain L which originally produced a high percentage of sarcomas in strain C_3H mice, now generally fail to produce tumors in strain C_3H mice unless these mice have been subjected to whole-body-x-irradiation before cell injection.

Methods employed: Strain C_3H mice are injected with cells of clone 929 and after a two week period are x-irradiated and challenged with an injection of 929 cells to see if immunity has developed as compared with control animals that received no prior injection. Tumors arising in x-irradiated animals are subinoculated into other strains of mice with different histocompatibility alleles to see if a detectable genetic change has occurred in the clone. Cells are also placed in cell-permeable diffusion chambers and grown in immunized and non-immunized mouse hosts.

Major findings: It has been found that clone 929 cells immunize or increase the resistance of the strain C_3H mouse to subsequent implantation of cells of clone 929. To a more limited degree clone 929 cells also increase the resistance of the strain C_3H mouse to subsequent implantation of cells of clone 1328. Tumors arising from clone 929 fail to grow in all other strains of mice tested. Clone 929 cells grown in the mouse in diffusion chambers that allow host cell penetration are reduced in numbers when grown in an immunized animal as compared with a non-immunized control animal. It is concluded that the clone 929 cells generally fail to grow in strain C_3H mice because they have become antigenic to the strain C_3H mouse and induce an immune reaction which tends to destroy them. The tumor cells were

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

for the strain C_3H mouse. Tumors of substrain III that have grown in x-irradiated mice were found on subinoculation to grow in all non-irradiated strain C_3H mice injected; furthermore, cultures of these tumors continued to produce approximately 100 percent tumors in non-irradiated mice. These tumors were found to immunize the strain C_3H mouse. Cells of substrain III grown in diffusion chambers in immunized and non-immunized mice showed some evidence of damage in immunized animals. Although the production of an immune reaction in the strain C_3H mouse may account for some of the failure of substrain III cells to produce tumors, there appear to be other factors involved that are under investigation.

Proposed course of project: One other experimental approach is being applied to a study of the differences in tumor-producing capacity of substrains III and VII and substrain III after one mouse passage. The capacity of substrain VII to induce immunity is also being investigated. A manuscript on this study is in preparation. Single cell isolations are being made from these substrains to test for homogeneity of the strains and to reveal new variants.

Significance to cancer research: This analyses of the behavior of culture strains that differ markedly in their ability to produce sarcomas in the strain C_3H mouse and that have transformed from normal to tumor-producing cells should provide some information as to the type of metabolic change that these cells have undergone in their transformation.

PROJECT REPORT FORM (Cont'd)

5. ACI-431 (C)
SERIAL NO.

6. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

The development of variations in transplantability and morphology within a clone of mouse fibroblasts transformed to sarcoma-producing cells in vitro. K.K.Sanford, G.D. Likely and W.R.Earle. J. Nat. Cancer Inst. 15:215-230, 1954.

7. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

PROJECT REPORT FORM (Cont'd)

NCI-431(c)

10. NCI-431(c) Hirst
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 29,656	7	7	14	2.3⅓	1.5⅓	3.8⅔	
FY 1956	$ 29,619	7	7	14	2.3⅓	1.5⅓	3.8⅔	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM

1. National Cancer Institute — 2. Biology LABORATORY OR BRANCH

3. Tissue Culture Section SECTION OR SERVICE — 4. LOCATION (IF OTHER THAN BETHESDA) — 5. NCI-431(3) SERIAL NO

Study of two strains of mouse liver epithelium originally from the same single cell.
PROJECT TITLE

Evans, V.J., Westfall, B.B. and Earle, W.R.
PRINCIPAL INVESTIGATOR(S)

Hawkins, N.M.
OTHER INVESTIGATORS

PROJECT DESCRIPTION : Objectives - To study two substrains, arising from a common clone of mouse liver epithelium. One of these strains is of high tumor producing ability the other is low in tumor production. The primary objective is to have available two such strains for comparing their metabolic and physiologic characteristics.

Methods employed: Cells cultured have been studied in vitro and have been continuously injected in both x-irradiated and non-irradiated C3H mice. The behavior of these cells in replicate culture has been studied for their response metabolically to increased amounts of dextrose.

Major findings: Mouse liver clone 1469 was originated from a single cell of strain 721 in April 1948. This strain 1469 in replicate cultures showed a higher content of glycogen when given a high concentration of dextrose than the content on a low concentration. Recently this clone cell strain has shown a low capacity for developing tumors on injection. Tissue cultures from one of these tumors developed a much enhanced capacity for its cells to give rise to tumors on re-injection into C3H mice.

Projected work: It is now planned to clone cells of this tumor strain so that 2 clone strains of liver epithelium will be available for experimental work. Studies will be made to learn if the second clone strain preserves its present ability to give rise to the same high incidence of tumors as the mixed strain that arose from the tumor. This work is necessary in order to have information on cell strains that will be used for metabolism studies.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Significance to Cancer Research: A comparative study of the metabolic and physiologic behavior of these two strains in Tissue Culture appears to be of significance in understanding the characteristics of transition from a cell strain able to give rise to practically no tumors to one able to give rise to a high percentage of tumors.

Comparison microcinematograph.

NCI-432

PROJECT REPORT FORM

1. National Cancer Institute
2. Biology
LABORATORY OR BRANCH

Tissue Culture
SECTION OR SERVICE
4.
LOCATION (IF OTHER THAN BETHESDA)
5. ZO1-432
SERIAL NO

Comparison Microcinematograph
PROJECT TITLE

Wilton R. Earle
PRINCIPAL INVESTIGATOR(S)

Other members of the section.
OTHER INVESTIGATORS

PROJECT DESCRIPTION: A three optical system has been developed and constructed in the NIH Instrument Shops. This is now undergoing final adjustments for use in the quantitative comparative study of various strains of malignant cells and of normal animal tissue cells. Attention is called to the fact that final adjustment and use of this instrument has been delayed more than a year now. Most of this delay has resulted from the fact that the development of the massive fluid suspension culture was considered so urgent and so significant that final adjustment on this camera was deliberately shelved in order to allow the fluid suspension culture to be pressed at full speed.

At the present time work is again returning to a normal rate of progress on the cinematograph. The editing machine has been rebuilt and has just been delivered from the Instrument Design Section. Final centering of lenses on the camera is now being completed. As soon as these technological points are completed, studies are planned on the following strains of cells: 1) Strain L fibroblast, to complete the standardization of this cell as one of 4 existing clone strains and the best documented of the 4. 2) Liver epithelium, clone 1469, an extraordinarily stable strain of normal mouse origin, the injections of which give rise to tumors in only 1/5 percent of their cases. 3) Liver epithelium, derived from 1469, now able to give rise to a higher percentage of tumors. (Evans, Westfall, Hawkins and Earle). The comparison of numbers 2 and 3 gives us our closest practical approach at present available to a morphologic comparison of a malignant cell type and the (apparently) normal cell from which it arises. 4) A clone fibroblast mouse strain able to give rise to sarcoma in 3% of its injections. 5) A fibroblast strain,

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

from the same single cell as (4), able to give rise to sarcoma in 97% of its injections. The morphology of the cells of these strains is radically different; their cinematographic comparison should give significant data on some of the changes which have occurred. 6) In the human skin the epithelium in cultures laterally decoheres, separates, and the cells become migratory, elongate, fibroblast-like. This phenomenon has greatly confused all recognition in skin tissue cultures. An attempt will be made to graphically demonstrate it. 7) Development of methods and equipment for quantitative comparative cinemicrography of animal tissue cells in culture.

10. M-432
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$8,374	1	1	2	1/2	1.0	1 1/2	
FY 1956	$8,410	1	1	2	1/2	1.0	1 1/2	

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Office of the Chief — SECTION OR SERVICE
4. Bethesda, Maryland. — LOCATION (IF OTHER THAN BETHESDA)
5. ZCl-500 — SERIAL NO

"Histogenesis and pathology of induced and spontaneous tumors of laboratory animals".
PROJECT TITLE

Dr. Harold L. Stewart
PRINCIPAL INVESTIGATOR(S)

Dr. Katharine C. Snell
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Project title: "A study of the histogenesis and pathology of induced and spontaneous tumors in laboratory animals."

Objectives: To gain knowledge concerning the etiological and pathogenic factors in the production of neoplasms in experimental animals and to make information obtained available to other investigators.

Methods employed:

1. A fascicle on transplantable and transmissible animal tumors is being prepared for publication in the Atlas of Tumor Pathology under the auspices of the Subcommittee on Oncology of the Committee on Pathology of the National Research Council. This fascicle is to include the history, transplantation behavior, detailed pathological description, and microphotographs of 50 of the most widely used animal tumors. (Dr. Lucia Dunham is collaborating on this project).

2. A large series of untreated rats of seven strains, males and females, breeders and non-breeders is being necropsied at various age intervals and subjected to detailed pathological examination in order to enumerate and classify spontaneous tumors, to study tissue changes with increasing age, and to furnish controls for present and future experiments.

3. Carcinogenic and non-carcinogenic compounds in different vehicles are introduced into various organs of experimental animals by means of injection or by being incorporated in food or drinking water. Animals employed are mice, rats, guinea pigs, rabbits, and monkeys.

4. A study of tumors occurring in the NHO strain of mice is being made. These mice are of interest because of the fact that their ancestors received a single dose of methylcholanthrene in an effort to produce an hereditary gastric carcinoma.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

9. PROJECT DESCRIPTION (Cont.)

Major findings:

1. Fascicle on Transplantable and Transmissible Animal Tumors: The manuscripts and microphotographs have been completed on all tumors; final correction and editing is in progress; many outside investigators have been consulted concerning recent experimental data on and the behavior of various tumors.

2. A total of 378 age control rats has been necropsied. Unusual tumors have been noted including a lung tumor, testicular tumors, carcinomas of the adrenal cortex, and a carcinc-sarcoma of the jejunum.

3. Results obtained from the transplantation of precancerous lesions of the glandular stomach of mice and rats and the intrahepatic injection of 9,10,dimethyl-1,2-benzanthracene are now being evaluated.

4. Various tumors in the NHO strain of mice have been studied, including tumors of the nervous system, lung, uterus, and of the reticulo-endothelial system.

Significance to Cancer Research: The knowledge gained from a study of neoplasms in laboratory animals should provide a basis for further work on the mechanism of carcinogenesis in the human being.

Proposed course of the project: It is expected that the Fascicle on Transplantable and Transmissible Animal Tumors will be ready for publication within the next few months.

Experiments dealing with carcinogenesis will be continued. In addition to those experiments mentioned above, an effort to produce pulmonary adenomatosis by feeding 1,2,5,6-dibenzanthracene to DBA mice will be continued, and an experiment designed to produce rhabdomyosarcoma in the rat will be undertaken.

10. M-5110
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$14,591	2	2	4	1	1	2	0
FY 1956	$14,593	2	2	4	1	1	2	0

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

To my knowledge no similar research is in progress elsewhere.

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Pathology Section — SECTION OR SERVICE
4. Bethesda, Maryland — LOCATION (IF OTHER THAN BETHESDA)
5. ZCL-501 — SERIAL NO

6. Investigations in the Geographic Pathology of Cancer
PROJECT TITLE

7. Project 1) Dr. Harold L. Stewart, Dr. Harold F. Dorn, Dr. Lucia J. Dunham
PRINCIPAL INVESTIGATOR(S)
Project 2) Dr. Lucia J. Dunham, Dr. Harold F. Dorn
Project 3) Dr. Lucia J. Dunham

Project 1) Dr. John H. Edgcomb, Dr. Louis B. Thomas and Dr. C. Harold Steffee;
OTHER INVESTIGATORS
*Refer to Mr. Haenszel's report - Biometry Section, Epidemiology Branch

8. PROJECT DESCRIPTION

Project 1): Field studies on female genital cancer in New York City and Israel.
Project 2): Techniques in the geographic pathology of cancer.
Project 3): Experimental work related to the geographic pathology of cancer.

Objectives:

Project 1) Studies on uterine cancer (uterine cervix and corpus) in Jewish, non-Jewish white and Negro patients in New York City, and on other female genital cancer in patients in Israel, are being made with a view to facilitating further understanding of the differences noted in these groups.

Project 2) It is hoped to increase the value of comparative studies in geographic pathology by a scholarly study of present techniques and offering suggestions for improving and standardizing these.

Project 3) Means are being devised for testing in the laboratory etiologic factors suggested by geographic pathology studies.

Methods Employed:

Project 1) The study is being pursued by a group of trained investigators in New York City and Israel. Coding of the results and a statistical analysis are being carried out at the National Cancer Institute. Three parallel aspects of the study are pursued (1) Obtaining records of all uterine cancer cases diagnosed in the areas indicated,

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT DESCRIPTION (Cont'd)

with information on the patient's color, religion, age, exact diagnosis, etc. (In Israel, breast cancer and other female genital cancer have also been investigated); (2) Interviewing all uterine cancer patients possible, and an equal number of control patients with a view to establishing any factors in the patient's past history worthy of further study in elucidating uterine cancer differences in incidence; (3) Pathologic study of material from all possible cancer cases in the above studies.

Project 2) Preparation of an exhibit and accompanying leaflet on Techniques in the Geographic Pathology of Cancer, with world maps on relative cancer frequencies.

Project 3) Initiating this work with studies on oral cancer. It is planned to keep suspected agents in chronic contact with the hamster pouch mucosa.

Major Findings:

Project 1) Corpus cancer is more frequent than cervix cancer in Jewish women, but because the latter type is less frequent rather than because corpus cancer is more frequent. The New York City and Israel data agree in these respects. In non-Jewish white women cervix cancer appears to be at least twice as frequent as corpus cancer, and cervix cancer appears to be very much higher in Negro women. The coding of the New York City questionnaires exclusive of those from Negro patients is nearly complete, Israel interviews are complete, and pathology studies from both areas are about half complete.

Project 2) None. Interest has been expressed by geographic pathologists.

Project 3) None. The work is still in its initial stages.

Significance to Cancer Research:

Project 1) As female genital cancer and especially uterine cancer are the most common causes of illness and death from cancer in women, any knowledge of contributing causes will help in the control of these diseases.

Project 2) Anything that will contribute to more uniform reporting of studies in geographic pathology will contribute to their reliability and value.

Project 3) Answers to some of the questions posed in geographic pathology studies may be more readily and promptly answered in the laboratory than by population studies.

10. [illegible]
SERIAL NO.

11. MAN YEARS DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$ 24,519	6	2	8	1½ yrs	2 yrs.	3½ yrs.	0
FY 1956	$ 24,549	6	2	8	1½ yrs	2 yrs.	3½ yrs.	0

12. BUDGET ACTIVITY:

RESEARCH ☑ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Project 1) Mr. W. M. Haenszel, Dr. Harold F. Dorn
Project 2) Dr. Harold F. Dorn
Project 3) Dr. Marvin Burstone

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project does not to our knowledge parallel research done elsewhere in the Public Health Service

PROJECT DESCRIPTION (Cont'd)

Proposed Course:

Project 1) It is planned to continue interviewing of Negro patients for 2 years. Pathology studies will be completed, and coding, punching, and analysis of questionnaires will be completed.

Project 2) It is planned to keep up to date a critical appraisal of this field.

Project 3) As stated for 3) under Methods Employed.

7a1-51
SERIAL NO.

PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

The material of the exhibit on Techniques in the Geographic Pathology of Cancer (Project 2), adapted for publication with the Proceedings of the Vth International Conference on Geographic Pathology; to appear in the Schweiz. Ztschr. f. allg. Path. u. Bakt.

HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None.

PROJECT REPORT FORM

1. National Cancer Institute	2. Laboratory of Pathology		
INSTITUTE	LABORATORY OR BRANCH		
Office of the Chief	4. Bethesda, Maryland .	5Z01-502	
SECTION OR SERVICE	LOCATION (IF OTHER THAN BETHESDA)	SERIAL NO.	

"Histopathology and histogenesis of neoplasms of the skin."
PROJECT TITLE

Dr. John H. Edgcomb
PRINCIPAL INVESTIGATOR(S)

None
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Project Title: "Histopathology and histogenesis of neoplasms of the skin".
Objectives: To study available material relating to the pathologic changes in the skin of man and experimental animals and to perform experiments designed to afford understanding of the derivation of tumors from dermal and epidermal structures, the stages and patterns of tumor formation, and the behavior of various tumors as a function of their derivation and structure.
Methods employed:

I. Collaborative studies with material accumulated on skin tumors.
   (a) Several experiments with a transplantable, keratin-producing cystic tumor of the skin of the strain C mouse have been performed.
   (b) A method for staining the pituitary gland of the mouse has been perfected in Mr. Albrecht's laboratory and will be applied to hairless mice secured from Dr. Deringer.
   (c) The study of samples from the skin of necropsied zoo animals is continuing.
   (d) Several hundred tumors from the experiments of Dr. Andervont on the effect of strain on tumor yield in mice subjected to a standardized skin painting procedure are being classified, specially stained and analyzed.

II. Experiments conducted by the investigator.
   (a) An attempt to produce malignant melanomas in guinea pigs by repeated skin painting with dimethyl-benzanthracene.
   (b) Experiments to characterize the leukemoid reaction induced by the transplantation of the Bryant tumor of strain C mice.
   (c) The maintenance of a colony of hairless mice.

III. Studies of tumors in man.
   (a) I have assumed a considerable share of the surgical pathology and necropsy services in the Clinical Center.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

9. PROJECT DESCRIPTION: (Cont.)

Major Findings: b) At present, due to moving of laboratories into the Clinical Center and to a low degree of fecundity, the hairless mice have not increased in number. Studies of the pituitary glands of a few animals by several methods have resulted in the choice of a method which should allow a quantitative assessment of the numbers of cells of different types present.

(a) An experiment on the leukemoid reaction secondary to the suvcutaneous transplantation of the Bryant tumor has led to the following conclusions:
(1) The reactions are severe; cell counts of 500,000 mm. have been observed 8-12 weeks after transplantation.
(2) The cells involved are polymorphonuclear neutrophils.
(3) The reaction appears as the tumor becomes cystic and necrotic and disappears within three days of the excision of the tumor.

(c) No major results.

(d) Dr. Andervont is reporting the results.

II.

(a) Seven of forty-seven guinea pigs now have small tumors.

(b) See C-I-a.

(c) See C-I-b.

III.

(a) I have performed nine necropsies and have either described or checked out about two-thirds of the accessioned surgical pathology material received in the last four months of the year. This activity has caused me to discontinue my studies at the Armed Forces Institute of Pathology.

(b) Due to the temporary illness of Dr. Steffee, I have contributed some time to the diagnosis of cases and to the administration of some parts of the study on uterine cancer at present being conducted by the Section of Geographical Pathology.

10. [illegible]
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$17,2[illegible]	1	2	3	1	1 ½	2½	0
FY 1956	$17,5[illegible]	1	2	3	1	1	2	0

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not to my knowledge

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute INSTITUTE	2. Laboratory of Pathology LABORATORY OR BRANCH		
3. Cancer Induction & Pathogenesis SECTION OR SERVICE	4. Bethesda, Maryland. LOCATION (IF OTHER THAN BETHESDA)	Z1CI-503 SERIAL NO	

"Morphology of neoplasms in laboratory animals(With particular reference to leukemia in mice)."
PROJECT TITLE

Dr. Thelma B. Dunn
PRINCIPAL INVESTIGATOR(S)

None
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Project title: "Morphology of neoplasms in laboratory animals (With particular reference to leukemia in mice)".

Objectives: To recognize and diagnose various neoplasms appearing in laboratory animals in order to understand significance, and to make this knowledge available to others engaged in cancer research. Special emphasis is placed on neoplasms of reticular tissue.

Methods employed: Control animals and animals bearing spontaneous or induced neoplasms are autopsied, and histologic studies are carried out. Special studies are made of the blood forming organs, including study of peripheral blood. Much of the work is collaborative, consisting in examination of animals used in a variety of experiments. Collections of tumors from other laboratories in the United States are examined.

Major findings: The morphology of many tumors has been described. The significance of several types as expressions of the neoplastic stimulus have been described.

Significance to Cancer Research: Since the neoplasm is the end point in many experiments, it is important that these neoplasms can be recognized and accurately diagnosed. Information on the comparative pathology of neoplasms is also important, since the different expressions of the neoplastic process may give some clue as to the factors which operate to produce them.

Proposed course of project: To continue observations on a variety of neoplasms in laboratory animals, always relating them to the broader problem of cancer in man. To describe and publish details regarding the normal and pathologic anatomy of laboratory animals, so they can be used to greater profit in experiments, especially those relating to cancer. (Note: - Much of the work is as a Consultant or Collaborator with other members of the Staff of the National Cancer Institute who have problems involving pathology.)

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT RENEWAL FORM (Cont'd)

10. [illegible]

11. [illegible]:

	ESTIMATED EXPENDITURES	PROFESSIONAL PERSONNEL			MAN YEARS			PATIENT DAYS
		PROF.	OTHER	TOTAL	PROF.	OTHER	TOTAL	
FY 1955	[illegible]	1	2	3	1	1-1/3	2-1/3	0
FY 1956	[illegible]	1	2	3	1	1-1/3	2-1/3	0

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not to my knowledge.

PROJECT REPORT FORM (Cont'd)

15. 304-519
INITIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

1. Dunn, T. B.: Normal and pathologic anatomy of the reticular tissue in laboratory mice, with a classification and discussion of neoplasms. J. Nat. Cancer Inst. 14: 1281-1433, 1954.

2. Dunn, T. B.: Importance of morphology in inbred strains of mice. J. Nat. Cancer Inst. (in press). To be published with a Symposium given at the Jackson Memorial Laboratory, Bar Harbor, Maine, commemorating the twenty-fifth anniversary of the founding of the laboratory.

3. Andervont, H. B., and Dunn, T. B.; Transplantation of hepatomas in mice. J. Nat. Cancer Inst. (in press). To be published in a Symposium on Hepatomas held at Arden House, New York, October, 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

1. Invited to give talk on "Importance of morphology in inbred strains of mice" (see publications) in honor of Dr. Elizabeth Fekete, who was completing 25 years at the Jackson Memorial Laboratory.

2. Invited to lecture (as a member of the faculty) on "Neoplasms in laboratory rodents", in a course on the Pathology of Diseases of Laboratory Animals, given at the Armed Forces Institute of Pathology, Dec. 6-10, 1954.

PROJECT REPORT FORM

National Cancer Institute 2. Laboratory of Pathology
INSTITUTE LABORATORY OR BRANCH

Cancer Induction & Pathogenesis 4. Bethesda, Maryland . 57C1-504
SECTION OR SERVICE LOCATION (IF OTHER THAN BETHESDA) SERIAL NO

"The electron microscopy of normal and pathologic tissues and tissue components"
PROJECT TITLE

Dr. William G. Banfield
PRINCIPAL INVESTIGATOR(S)

None
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Project title: "The electron microscopy of normal and pathologic tissues and tissue components".

Objectives: The objectives of this program are to establish a pattern of ultramicroscopic morphology for certain neoplasms and related normal and pathologic tissue and to follow the leads obtained that appear most promising.

Methods employed: Ultra thin sections are made from tissue obtained from human surgical and autospy specimens. These are studied with the electron microscope and their electron microscopic histology described. Other methods of preparation including homogenation and freeze drying complete shadow casting will be employed. Since these studies will deal with connective tissues as one aspect, related experiments involving chemical; physical chemical and biological methods will be employed.

Major findings: The project will not be underway until July 1954.

Significance to Cancer Research: The program will enable a study of alterations of ultrastructure in and about the neoplastic cell by direct visualization, such alterations heretofore have been either inaccessible or accessible only by indirect methods. In addition, such possible etiologic agents of the size of viruses would come into the range of visibility.

Proposed course of project: The results obtained from the methods employed will influence the direction of subsequent studies.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Ncl-504
SERIAL NO.

BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$9,325	1	—	1	1	-	1	0
FY 1956	$14,523	1	1	2	1	1	2	0

BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Laboratory of Pathology
LABORATORY OR BRANCH

3. Cancer Induction & Pathogenesis
SECTION OR SERVICE

4. Bethesda, Maryland
LOCATION (IF OTHER THAN BETHESDA)

5. NCI-505
SERIAL NO

"Veterinary Pathology"
PROJECT TITLE

Dr. L. H. Lyestone
PRINCIPAL INVESTIGATOR(S)

None
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Project title: "Veterinary Pathology"

A. The behaviour of transplantable osteogenic sarcomas in C3H mice

Objectives: To test the influence of growth sites on the capability of these transplantable tumors to produce bone. (When transplanted routinely to the subcutaneous tissues, they lose this capacity in 3-5 generations.) Also to test the influence of various exogenous agents on bone production (Vitamin D, ultra-violet, and estrogenic hormones.)

Methods: To test the influence on site of growth, tumors are transplanted into skeletal muscle, intracranially, intraperitoneally, and near injured periosteum.

Major findings: Two cell types of the tumor have been carried among the five transplantable tumors investigated: the cord-cell type, which metastasizes readily and produces spicules of bone both in the transplant and in metastases, and the spindle-cell type, which metastasizes terminally. Both of these tumors produce more bone when transplanted into the muscle of the leg than in any other site. Exogenous agents so far have had no effect on the bone-producing capacity of the tumors. Selection of tumor for transplantation, from the soft periphery of the tumor usually produces a fast-growing, non-bone producing tumor while tissue selected close to the bone portion produces a slower-growing tumor with tendency to bone production, but is slower to metastasize than the former. Consistency of result has not been entirely uniform.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Significance to cancer research: The variation in functional activity of tumor cells (their ability to act like the normal parent cell) is one of the most perplexing problems in cancer research. Since bone tumors have not been investigated to any degree in this regard, it is hoped that this investigation will not only prove a valuable tool for future study, but will provide some information on their functional activity.

Proposed course of project: This project is now completed and the results are being analyzed in view of publication. The tumor strains will be maintained in the laboratory for possible future experiments.

B. Bone tumor carcinogenesis

Objective: To test the capacity of intravenous Beryllium oxide to produce osteogenic sarcoma in dogs.

Methods: 200 milligrams of beryllium oxide were injected intravenously in a single injection into 8 dogs. These animals have now been under observation for over a year.

Major Findings: One animal has died (Approximately 1 year following the injection) and autopsy revealed multiple granulomatous lesions in the lymph node, spleen, bone marrow, lungs and liver. Refractile crystals, which may be beryllium, were observed in the granulomata. No evidence of bone cancer was seen. The remaining 7 animals are still in good health after 2 years.

Significance: Providing osteogenic sarcomas are produced in dogs (as has previously been done in rabbits), it is felt that a more suitable experimental animal for surgical and laboratory procedures will have been demonstrated.

C. The incidence of tumors in captive wild animals

Objectives: To perform autopsies on animals dying at the National Zoological Park, examine these for cancerous lesions, and prepare tissues for comparative microscopic study with human cancer.

Methods: A thorough autopsy, usually including the C. N. S., is performed, and the tissues prepared in preserving fluids for later sectioning and microscopic examination. Wet tissues and slide are retained in storage for future reference by the staff.

Major Findings: The incidence of cancer in a selected captive wild animal population. The types of cancer are quite similar but with notable exceptions, i.e. the lack of cancer in the gastrointestinal tract.

Significance to cancer research: The comparative histology of spontaneous lesions in animals provides, through analogy, a background of information which is valuable for the interpretation of experimentally produced lesions in laboratory animals. Through analysis of such spontaneous lesions many of which are in less complex form of animal life, reasoning as to their inception is sometimes made easier.

Dr. W. H. Lyncstone

F. Cancer in dogs

To diagnose and follow-up spontaneous tumors in dogs through clinical records, autopsy material and biopsies sent in by mail or received through personal contact with local veterinarians.

Methods: Pet tissues are received and processed in the laboratory and diagnoses are submitted to the contributor. This is done in return for clinical histories and annual follow-ups on biopsied cases from the contributors. Approximately 75 tissue biopsies and autopsy cases are received per year.

Significance to cancer research: Generally, cancer in dogs closely resembles human cancer both histologically and biologically; however, certain important differences have been noted which emphasize that histological evaluation of certain tumors is not consistent with experience in human cancer of similar type. Since there are some important biological variations in cancer among dogs, evaluation of experimentally produced tumors in dogs is more secure when a sound knowledge of their natural behavior is known.

0. NCI-505
SERIAL NO.

1. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$17,224	1	1½	2½	1	1½	2½	
FY 1956	$17,2[illegible]	1	1½	2½	1	1½	2½	

2. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

3. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not to my knowledge

15. ncl-525
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Habermann, Robert T., Fletcher P. Williams, and Willard H. Eyestone: Spontaneous Hepatomas in Two Woodchucks and a Carcinoma of the Testis in a Badger. From the Journal of the American Veterinary Medical Association, Vol. 125, No. 931, Oct., 1954, pp. 295-298.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Office of the Director, NCI — SECTION OR SERVICE
4. Bethesda, Maryland — LOCATION (IF OTHER THAN BETHESDA)

5. ZCI-JA-6 — SERIAL NO

Pulmonary tumor project
PROJECT TITLE

Dr. C. Harold Steffee
PRINCIPAL INVESTIGATOR(S)

None
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objectives:

1. To gain basic information on biologic aspects of cancer, using pulmonary tumors as a tool in this endeavor.
2. To gain information regarding the etiology and pathogenesis of human lung cancer, and to determine the carcinogenicity of various substances suspected as etiologic agents in human pulmonary cancer. This requires finding a method for producing similar tumors in animals.

Methods:

1. Biologic studies have been done using tumor transplantation and genetic techniques.
2. The search for an experimental neoplasm similar to human bronchogenic carcinoma has used threads coated with methylcholanthrene, embryo transplantation techniques, and the intratracheal administration of beryllium, the last in collaboration with Dr. A. W. Hilberg,(These are the major techniques by which bronchogenic tumors have allegedly been produced experimentally). Other studies have employed inhalation or intratracheal administration of chronates, (in collaboration with Dr. Anne M. Baetjer, Johns Hopkins University) and the preparation and intratracheal administration of cigarette smoke condensates. Histochemical techniques have been used on human trachea and bronchus.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT DESCRIPTION (Cont'd)

Major findings:

1. Histological findings on nearly all of the experiments have been delayed by the illness of the principal investigator for the past 7 months. Experiments on the relation of size of tumor to biological behavior have been completed and the gross data are in the hands of the statistician. It is tempting to record the gross results, but perhaps more prudent to wait until microscopic studies are completed. In a study of the sarcomatous transformation of the mouse pulmonary carcinoma, approximately 19 tumors are now in the first to ninth transplant generations, but complete sarcomatous transformation has not yet occurred in any of these tumors.

2. Insertion of hydrocarbon coated threads in the submucosa of the trachea of mice has been unsuccessful. No tumors have been produced in rats, hamsters, or guinea pigs with this technique, although about half of these animals are still living. Carcinogen-coated threads inserted through the lungs have produced tumors (sarcomas) in mice as reported last year. Three of four similarly treated hamsters have shown tumors at autopsy. None of the rats or guinea pigs have shown tumors, but a few rats and most of the guinea pigs are still alive. With embryo lung transplant techniques, using methylcholanthrene as a carcinogen, 7 sarcomas, 1 squamous cell carcinoma, and 2 adenocarcinomas have been produced in a total of 25 such transplants. While this group is too small to have any statistical significance, it is interesting to note that the only squamous neoplasm was obtained with a transplant of the periphery of the embryonic lung, whereas transplants of lung hilus, main bronchus, or trachea failed to produce such tumors. Intratracheal beryllium has failed to produce tumors to date, although it is perhaps too early to expect them. About 55 of the original 150 mice and 23 of the original 100 rats remain alive. The experiments at Johns Hopkins have to date failed to produce bronchogenic carcinoma in mice, rats, guinea pigs, or rabbits. Cigarette tar experiments were begun this year. Tar was prepared from unfiltered cigarette smoke, as well as from smoke filtered through silica gel, or through tobacco. Paradoxically, the yield of tar was essentially the same, whether or not filters were used. When the tar was administered to mice intratracheally, it was found that the highest non-lethal dosage was about 0.5 mg. Since these injections can only be given about every 4 weeks, a mouse would receive only 6.5 mg. of tar per year with this technique, and this dosage would not constitute an adequate test of the carcinogenicity of this material. It was thought that nicotine might be the most toxic component of these tars, so an attempt was made to desensitize a group of mice to nicotine. After 11 weeks, only 20 of the original 124 mice were still alive, and these animals showed no increase in tolerance to the cigarette tar. In addition, 13 embryo lung transplants were performed using cigarette tar as a "carcinogen". To date, no tumors have resulted and 11 animals are still living. While it is too early to evaluate this experiment, it is approaching the point of showing a significant lack of carcinogenicity of this tar. Histochemical studies on human material have been un-

PROJECT DESCRIPTION (Cont'd)

rewarding to date, because of autolysis in the two cases studied.

Significance to Cancer Research:
The study of the relationship of tumor size to transplantability is a problem in the fundamental nature of malignancy. Definition of the exact point at which benignity becomes malignancy would sharpen the focus for further biological and biochemical studies. Study of the origin of sarcoma cells is likewise of fundamental importance. If they are found to be derived from host stroma, this would be a demonstration of the induction of malignancy by another malignancy, a new concept in cancer research which would have far-reaching importance if true. The other experiments performed in this project represent a more direct attack on the human cancer problem. Bronchogenic carcinoma is now in many areas the commonest malignancy of men, and seems to be increasing in frequency. Many laboratories throughout the country are engaged in the search for etiologic agents but all of this work is seriously hampered by the lack of a suitable test object. Thus we have placed much emphasis on trying to produce an analagous tumor experimentally. Simultaneously we have studied the effects on experimental animals of two substances which have been implicated in human neoplasia, namely cigarette tars and chromates. Though these are but two of the many suspected etiologic agents, they at least represent a part of the attack on the vast problem of human lung cancer.

Proposed course of the project: Most of the experiments outlined above will be continued as indicated. The problem of the toxicity of cigarette tars will be explored further, and other techniques will be employed in an attempt to "desensitize" the animals. The toxicity of filtered smoke condensates will be compared with that of unfiltered products. Studies with embryo lung transplants will be expanded, and an attempt will be made to trace the cell of origin of the squamous cell tumors. More embryo lung transplants will be done with tobacco tars as a carcinogen. Also more mice will be given intrapulmonary carcinogen-coated threads, with refinements in technique designed to avoid production of subcutaneous sarcomas. Continuation of the collaborative experiment with Johns Hopkins is of course dependent upon renewal of the PHS grant (C603), to support the animal work, as only the pathologic studies are done in Bethesda. Another collaborative study with the Environmental Health Field Headquarters, Cincinnati, will be begun about March, 1955. This study will be concerned primarily with the water insoluble-acid soluble fraction, which has been implicated in the PHS monograph on chromates, and which has not been tested separately in the studies at Johns Hopkins. The Cincinnati group is preparing the fractions, and the intratracheal injections will be done here in Bethesda. Another collaborative study has been planned with Medical Director Randall B. Haas, U. S. Public Health Service Hospital, Manhattan Beach, to investigate other possible mechanisms of pulmonary carcinogenesis. These experiments will include intratracheal administration of carcinogenic hydrocarbons adsorbed on carbon particles to tuberculous and non-tuberculous animals, to determine the lipoid solvent effect of caseous necrosis. These experiments will also include studies on certain enzymes which have been reported to cause squamous metaplasia of bronchial epithelium in humans. The animal work in these experiments will be done at Manhattan Beach; we will perform the histopathological studies.

COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956. (Cont'd)

(Medical Director Randall B. Haas, Clinical Director) for experiments on aerosol enzymes and carcinogens adsorbed on carbon in tuberculous and non-tuberculous animals.
Dr. A. W. Hilberg, Laboratory of Pathology, National Cancer Institute, collaborator in intratracheal beryllium studies.

[illegible] (Cont'd)

[illegible]

[illegible]

	[illegible] EXPENDITURES	[illegible]	[illegible]	[illegible]	MAN YEARS			PATIENT DAYS
		PROF.	[illegible]	TOTAL	PROF.	[illegible]	TOTAL	
FY 1955	[illegible]	1	2	3	1	2	3	0
FY 1956	[illegible]	1	2	3	1	2	3	0

(This does not include animal care or histopathology services.)

12. PROJECT ACTIVITY:

RESEARCH ☑ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

PHS Grant C003, providing funds to Dr. Anne M. Baetjer, Department of Environmental Medicine, The Johns Hopkins University, 615 N. Wolfe Street, Baltimore, Maryland, for experiments on inhalation and intratracheal injections of carbonates.
Occupational Health Field Headquarters, 10th Broadway, Cincinnati, Ohio, (Basic I Director S. Clark Cooper, Chief) for experiments on the water insoluble-acid soluble chromate fraction.
U. S. Public Health Service Hospital, Manhattan Beach, Brooklyn, New York

14. IF THIS PROJECT RESEARCH, OPERATIONS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No similar research known to be in progress elsewhere.

[illegible]

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induction & Pathogenesis — SECTION OR SERVICE
4. Bethesda, Maryland — LOCATION (IF OTHER THAN BETHESDA)
5. NC/-507 — SERIAL NO

6. "Cytological effects of X radiation on tissue of mouse".
PROJECT TITLE

7. Dr. Ross C. MacCardle
PRINCIPAL INVESTIGATOR(S)

8. Dr. Charles C. Congdon
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project title: "Cytological effects of X radiation on tissue of mouse".

Objectives: Cytological effect of X irradiation on tissues of mouse (in collaboration with Charles C. Congdon of Radiation Branch, NCI): We have attempted to secure a description of the basic cytological changes in mitochondria, minerals, basophilic substances, Golgi apparatus, etc., following X irradiation of normal tissues, such as liver, bone marrow, spleen, thymus, islets of Langerhans, anterior lobe of the pituitary gland, carotid body, lung, etc. So far we have found an alteration of the mitochondria in the liver, - and believe it is related to metabolism of phospholipids.

PROJECT REPORT FORM (Cont'd)

10. MCI-537
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$4863	2	1	3	1/3	1/3	2/3	0
FY 1956	0	—	—	—	—	—	—	0

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not to my knowledge

15. NCI-537
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

"Mitochondrial changes in hepatic cells of X irradiated mice" by Ross C. MacCardle and Charles C. Congdon, submitted in 1954 to Editors of American Journal of Pathology.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induction & Pathogenesis — SECTION OR SERVICE
4. Bethesda, Maryland — LOCATION (IF OTHER THAN BETHESDA)
5. ZCL-508(C) — SERIAL NO

6. "The skin of zinc deficient animals" — PROJECT TITLE

7. Dr. Ross C. MacCardle — PRINCIPAL INVESTIGATOR(S)

8. none — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project title: "The skin of zinc deficient animals"

Objectives: Effects of zinc deficiency on the skin and pancreas. I have some evidence that zinc-deficient diet begun in pregnancy halt the formation of the mesodermal hair papilla but not the down-growth of epidermal hair buds in new-born mice. The effect on the islet cells of the pancreas its not clear. I wish to study the pancreas and zinc content of hairless mice, rats, pigs, white horses, cattle and men.

10. NCJ-5088(c)
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 4,863	1	1	2	1/3	1/3	2/3	0
FY 1956	$4,865	1	1	2	1/3	1/3	2/3	0

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not to my knowledge

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute	2. Laboratory of Pathology			
INSTITUTE	LABORATORY OR BRANCH			
3. Cancer Induction & Pathogenesis	4. Bethesda, Maryland		5 Jnc-1-508(4)	
SECTION OR SERVICE	LOCATION (IF OTHER THAN BETHESDA)		SERIAL NO	

"The cytology of normal human skin and lesions such as cutaneous tumors and abnormal growths of hair."
PROJECT TITLE

Dr. Ross C. MacCardle
PRINCIPAL INVESTIGATOR(S)

E. J. Van Scott
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Project title: "The cytology of normal human skin and lesions such as cutaneous tumors and abnormal growths of hair."

Objectives: In collaboration with Dr. Van Scott: A Study of the Cytology (sulfhydryls, tonofibrils, and other elements) of cutaneous tumors, sclerodermatous lesions of skin, Bowen's disease, and the nature of "clear cells." This work concerns the relationship of precancerous changes and the development of tumors.

PROJECT REPORT FORM (Cont'd)

10. NC1-508(L)
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$4563	2	1	3	1/3	1/3	2/3	10
FY 1956	$4865	2	1	3	1/3	1/3	2/3	10

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not to my knowledge

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute	2.	Laboratory of Pathology		
INSTITUTE		LABORATORY OR BRANCH		
3. Cancer Induction & Pathogenesis	4.	Bethesda, Maryland	5. NCI-509(L)	
SECTION OR SERVICE		LOCATION (IF OTHER THAN BETHESDA)	SERIAL NO	

"Cytology of thymus gland"
PROJECT TITLE

Dr. Ross C. MacCardle
PRINCIPAL INVESTIGATOR(S)

Dr. Charles C. Congdon
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Project title: "Cytology of thymus gland".

Objectives: Cytology of thums form fetal development to senility with a view toward a study of effects of X rays, and an evaluation of cytology of thymus of high-leukemic and low-leukemic organisms.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

10. Rel-509(c)
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$4,883	2	1	3	1/3	1/3	2/3	0
FY 1956	0	—	—	—	—	—	—	0

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not to my knowledge

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Laboratory of Pathology
LABORATORY OR BRANCH

3. Cancer Induction & Pathogenesis
SECTION OR SERVICE

4. Bethesda, Maryland
LOCATION (IF OTHER THAN BETHESDA)

5 NCI-589(L)
SERIAL NO

6. "Writing of a Monograph on "The Tumor Cell"
PROJECT TITLE

7. Dr. Ross C. MacCardle
PRINCIPAL INVESTIGATOR(S)

8. none
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project title: "The Tumor Cell"

Objectives: I am writing by invitation a short manuscript on "The Tumor Cell: Characteristics of malignant protoplasm" to be included in a series of volumes edited by Heilbrunn and Weber entitled "An International Treatise on Physiology of Protoplasm."

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

0. Ped-509(B)
SERIAL NO.

1. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$3,108	1	—	1	1/3	—	1/3	0
FY 1956	$3,092	1	—	1	1/3	—	1/3	0

2. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

3. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not to my knowledge

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induction & Pathogenesis — SECTION OR SERVICE
4. Bethesda, Maryland. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-809(C) — SERIAL NO

6. "Cytology of polycythemia" — PROJECT TITLE

7. Dr. Ross C. Mac Cardle — PRINCIPAL INVESTIGATOR(S)

8. none — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project title: "Cytology of polycythemia"

Objectives: I wish to study the effect of copper and cobalt deficient diets on polycythemia induced at high altitudes.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

10. Ref-509(a)
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$3108	1	—	1	1/3	—	1/3	0
FY 1956	$3092	1	—	1	1/3	—	1/3	0

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not to my knowledge

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Laboratory of Pathology
LABORATORY OR BRANCH

Cancer Induction & Pathogenesis
SECTION OR SERVICE

Bethesda, Maryland
LOCATION (IF OTHER THAN BETHESDA)

5. ZCA-509(C)
SERIAL NO

"Cytology of human and animal tumors"
PROJECT TITLE

Dr. Ross C. MacCardle
PRINCIPAL INVESTIGATOR(S)

none
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Project title: "Cytology of human and animal tumors"

Objectives: Topography of intracellular minerals of human and animal tumors:
I have studied the minerals of human lesions such as those of basal-cell cutaneous tumors, Bowen's disease, squamous-cell carcinoma of skin, leukoplakia, carcinoma of the tongue, arsenical exfoliative dermatitis, xeroderma pigmentosum, scleroderma, an Islet-cell tumor of the pancreas, and a tumor of the cortex of the adrenal gland. This type of study is being done in order to compare the distribution of minerals with that of "normal" human tissues. I have also used part of this tissue for a future study of the cytology (mitochondria, etc.,) of human tumors. Murine tumors include Sarcoma 37, hepatoma of mouse and rat, and testicular tumors.

Research work planned for future:

1. Cytology of human tumors.
by light microscopy, incineration, and (in collaboration with Dr. Banfield) electron microscopy.

(PUT ADDITIONAL SHEETS AS NECESSARY FOR CONTINUATION)

10. Dent-5098(1)
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	4403	1	1	2	½	½	1	0
FY 1956	4403	2	1	3	½	½	1	0

12. BUDGET ACTIVITY:

RESEARCH ☑ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. OTHER U.S. UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not to my knowledge

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induction & Pathogenesis — SUBJECT OR SERVICE
4. Bethesda, Maryland — LOCATION (IF OTHER THAN BETHESDA)
5. ZCP-5096(C) — SERIAL NO

6. "Mitochondria in situ in normal and malignant cells" — PROJECT TITLE

7. Dr. Ross C. MacCardle — PRINCIPAL INVESTIGATOR(S)

8. None — OTHER INVESTIGATORS

PROJECT DESCRIPTION

Project title: "Mitochondria in situ in normal and malignant cells".

Objectives: "elation of mitochondria and Nadi oxidase granules: I have found that the mitochondria can be dislocated through the cytoplasm to one end of the cell, leaving the Nadi oxidase granules behind in the mitochondria-free cytoplasm at the opposite pole of the cytoplasm, - after ultracentrifugation with Beams' high-speed air-driven centrifuge. I have studied this phenomenon in normal liver of mouse, epididymis of mouse and of the duck and hepatoma of mouse.

10. Ncl-5796(c)
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$4863	1	1	2	1/3	1/3	2/3	0
FY 1956	$4865	1	1	2	1/3	1/3	2/3	0

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not to my knowledge

15. NCI-5021(a)
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Manuscript in preparation.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induction & Pathogenesis Section — SECTION OR SERVICE
4. Bethesda, Maryland — LOCATION (IF OTHER THAN BETHESDA)
5. ZIC/-5788(C) — SERIAL NO

6. "Cytology of Sarcoma 37" — PROJECT TITLE

7. Dr. Ross C. MacCardle — PRINCIPAL INVESTIGATOR(S)

8. None — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project title: "Cytology of Sarcoma 37".

Objectives: This transplantable spontaneous tumor was found in a mouse in 1906, and is very widely used in experimental work. I have found that its Golgi apparatus varies very greatly in form, being distributed throughout the cytoplasm as dictyosomes (rods) at 3 days following transplantation when the transplant is not fully vascularized, and in compact as a small juxtanuclear network at 6 days after transplantation when it is fully vascularized highly active in respect to rate of growth and mitotic division. Its cells possess little iron and are rich in calcium and magnesium at 3 days after transplant, whereas at 6 days the tumor is rich in iron and relatively poor in calcium and magnesium. I believe that it grows best when transplanted in skeletal muscle such as that of the thigh. I have also studied the basophilic material (nucleoproteins) of this tumor in transplants derived from tumor mash left standing for different period of time after preparation from the donor.

SERIAL NO.

[illegible] DATA:

	ESTIMATED EXPENDITURES	PERSONNEL POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	[illegible]	1	--	1	1/3	--	1/3	0
FY 1956	$5,[illegible]00	1	--	1	1/3	--	1/3	0

[illegible] REPORT ACTIVITY:

RESEARCH ☑ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

[illegible] COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

[illegible] IF THIS IS A CONTINUATION OF [illegible], OR PARALLELS RESEARCH DONE ELSEWHERE [illegible] PUBLICATIONS [illegible], MACHINERY OR [illegible]

15. Z01-509(L)
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 19[illegible]:

Manuscript in preparation.

HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR [illegible] 19[illegible]:

None

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Laboratory of Pathology
LABORATORY OR BRANCH

3. Cancer Pathology Section
SECTION OR SERVICE

4. Bethesda, Maryland
LOCATION (IF OTHER THAN BETHESDA)

5. ZCL-570
SERIAL NO

"The Biologic mechanisms of malignancy in man, and their alteration by therapeutic agents, studied with sponge matrix tissue culture.
PROJECT TITLE

Dr. Joseph Leighton
PRINCIPAL INVESTIGATOR(S)

Mr. Ira Kline (Laboratory of Chemical Pharmacology).
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Project title: "The Biologic mechanisms of malignancy in man, and their alteration by therapeutic agents, studied with sponge matrix tissue culture."

Objectives:

1. Cultivate tumors from the operating room to observe the extent of differentiation in vitro as a supplementary aid to the evaluation of sections made of biopsy and autopsy material.

2. Develop stable subculture of human tumor cell lines whenever possible so that a variety of types of such cells may be available for study.

3. Study some of the mechanisms of the malignant process with combinations of tumor and normal tissue. Place emphasis on the factors which may alter the growth of the tumor into the normal tissue.

4. Establish a screening program in which chemical agents will be tested against many kinds of human cancer.

Methods employed: Sponge matrix methods for tissue culture will be used in this work.

PROJECT DESCRIPTION (Cont'd)

Major findings:

1. Tissue culture studies on the destructive invasion of normal tissues by cells of a human cancer (strain HeLa) have been extended. The tumor has been seen to invade the cells of the human uterine wall as well as into several embryonic tissues including heart, brain, lung and bone.

2. Variations of sponge matrix methods have been developed which permit the screening of chemical agents on tumor cells invading normal tissues.

3. In intimate collaboration with the Laboratory of Chemical Pharmacology the action of a chemical agent, #3022, a podophyllin derivative on combined cultures of normal and cancer tissues has been studied at screening levels as well as with methods permitting more detailed study.

Significance to cancer research: Proposed anti-cancer drugs may be tested in a situation where a human cancer is actively invading and replacing normal tissue. Invasive phenomena previously not observable under experimental conditions, may now be studied and the effects of drugs on the malignant process itself may be seen.

Proposed course of project:

1. In close collaboration with the Laboratory of Chemical Pharmacology, potential chemotherapeutic agents will be examined in systems where tumor cells are actively invading normal cells, and evidence of alteration of the malignant process will be sought.

2. Studies on the physiology of invasiveness will be extended.

3. The search for methods of growing human cancers readily in vitro will continue. Should the growth of human cancer in tissue culture be accomplished routinely, we may be able to aid in the selection of the drug of choice of the patient.

10. Inc1-510
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$21,886	2	2	4	1½	1½	3	0
FY 1956	$21,890	2	2	4	1½	1½	3	0

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No similar research is known to be in progress elsewhere.

15. Pcl-510
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

1. Leighton, J.; The growth patterns of some transplantable animal tumors in sponge matrix tissue culture. J. Nat. Cancer Inst. 15: 1954.

2. Leighton, J.: Studies on human cancer using sponge matrix tissue culture. I. The growth patterns of a malignant melanoma, adenocarcinoma of the parotid gland, papillary adenocarcinoma of the thyroid gland, adenocarcinoma of the pancreas, and epidermoid carcinoma of the uterine cervix (Gey's HeLa Strain), Texas Reports on Biol. & Med. (In press), 1954.

3. Leighton, J., and Kline I.: Studies on human cancer using sponge matrix tissue culture. II. The invasion of connective tissue by carcinoma (Strain HeLa). Texas Reports on Biol. & Med., (In press), 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

Talks, by invitation:

A. Gordon Cancer Conference, Sept. 2, 1954.

B. Oak Ridge National Laboratory, Nov. 18, 1954.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induction & Pathogenesis — SECTION OR SERVICE
4. Bethesda 14, Maryland — LOCATION (IF OTHER THAN BETHESDA)
5. ZA1-511 — SERIAL NO

6. "Effect of neoplastic diseases and of various stresses on the Pathophysiology of certain endocrine organs in man and in animals." — PROJECT TITLE

7. L. W. Nadel (see #13 for additional information) — PRINCIPAL INVESTIGATOR(S)

8. (see #13 for additional information) — OTHER INVESTIGATORS

9. **PROJECT DESCRIPTION**

Project title: "Effect of neoplastic disease and of various stresses on the pathophysiology of certain endocrine organs in man and in animals."

DESCRIPTION: The present project title includes two smaller projects previously described as follows:

(a) Role of ascorbic acid in the metabolism of tumor bearing, stressed and normal animals.

(b) Identification of steroid hormones in the tissues, blood and urine of stressed and tumor bearing animals.

The present project includes the above studies as well as the application of such studies to clinical medicine.

Objective: (1) To define the chemical structure, biologic nature and physical properties of specific corticosteroids and ketosteroids present in the tissues blood and urine of normal, stressed and tumor-bearing patients and guinea pigs.

(2) To establish methods for the quantitative measurement of such steroids as an aid in the study of the development of tumor growth and the course of the neoplastic disease in man and in guinea pigs.

(3) To correlate the pathophysiological findings with the morphological findings in the tissues in man and in guinea pigs.

(4) To study CHO metabolism as influenced by specific steroids in guinea pigs.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

9. Project Description (Cont.)

Methods: (The term stress is used in its widest concept for the purposes of this project, and includes, dietary deficiency and excesses as stresses, hormonal excesses and deficiencies as stresses, x-ray irradiation effects, stress by physical and microbial agents, carcinogens, and the effect of carcinoclastic and potentially carcinostatic drugs.)

Stressed animals, normal controls and tumor bearing animals are prepared biologically, and the clinical course followed. Tissues, blood, and urine are collected during the course of the observation period and at post-mortem examination, for chemical, histochemical and histopathological study. Extraction, purification and analysis are done by specific applications of chromatographic and spectrophotometric methods.

Major Findings:

a. A new series of corticosteroids with more polar (and less mobile) chromatographic properties than cortisone has been discovered in the urine of guinea pigs.
b. Five of these steroids have been crystallized and four have been identified.
c. The three strongest reducing steroids (of these five) have been studied in great detail in normal, stressed (see above) and tumor bearing guinea pigs, and an excretion pattern for these 3 steroids has been found in the urine of tumor bearing animals, that, in the animals studied appeared different from all other experimental animals, with the exception of animals exposed to 2000 r (whole body radiation). Over 300 animals have been studied.
d. Using the techniques found valuable in the extraction isolation studies in guinea pigs, one of these 3 steroids has been identified and isolated from the urine of man. This steroid, 6 β hydroxycortisol, is the first evidence that 6 β hydroxylation occurs in vivo in man.
e. Other studies on the role of ascorbic acid in metabolism have inidcated that scorbutic guinea pigs, despite a high circulating level of glucocorticoids, cannot deposit glycogen in their livers. This defect remains despite the administration of fructose, glucose, galactose or alanine.
f. Further evidence that ascorbic acid is not necessary for the manufacture of corticosteroids, come from the isolation of cortisol (in larger quantities than in controls) from the blood and urine of ascorbic acid deficient guinea pigs.
g. Additional evidence that adrenal steroids and adrenal ascorbic acid are not as intimately related metabolically as heretofor suggested lies in the finding of adrenal hypertrophy, and decreased cholesterol in the adrenals of stressed chicks (malaria), in the presence of normal or greater than normal adrenal ascorbic acid.

Significance to cancer research:

a. The presence of what appears to be a "tumor corticosteroid excretion pattern" in guinea pigs suggests that there may be a similar pattern in man.
b. The presence of compounds more polar than cortisol in the urine of man and of guinea pigs suggests that conclusions by other groups on the nature of the corticosteroid excretion in cancer must be reevaluated.
c. There is evidence that a complete series of corticosteroids in man has been inadvertently ignored by virtue of the fact that the commonly accepted procedures used for the extraction of steroids actually destroy these newly identified compounds.

9. Project Description (Cont.)

d. Though the procedures used in identifying these steroids are time consuming, it may be possible to follow the course of neoplastic disease by appropriate study.
e. Conclusions on the role of ascorbic acid can only be studied in primates and guinea pigs.

Proposed course:

a. A study of the specific excretion pattern of the steroids that are more polar than cortisone is under way in patients in the Clinical Center. Patients with neoplastic diseases are available in the cases currently being studied at the clinical research facilities of the NCI, while patients without neoplastic diseases are being made available from the cases being studied on the Psychosomatic service of the NIMH.

b. A continued study on the effect of var ous stresses (see methods above) is contemplated, on the guinea pigs.

c. The effect of various potentially therapeutic agents on the course of cancer in the guinea pigs is being studied with the hope of picking up alterations in the specific corticosteroid excretion patterns associated with carcinostasis.

d. A continued study on carbohydrate metabolism in the guinea pigs is being pursudd with the hopes of applying this fundamental knowledge to the in-vitro perfusion studies that are planned when facilities become available.

10. ZCI-511
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$9,928	1	1	2	½	1	1½	0
FY 1956	$22,776	2	2	4	(1)*½	1(2/3)*	2 1/6	60

*possibility of a professional visting or guest scientist.

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

In the PHS
D. Jane Taylor, Chemotherapy Section, Lab. Trop. Dis. NMI, PHS
Dr. A. S. Mulay, Lab. of Pathology, NCI, PHS
Mrs. Wodinsky and Mr. Saslaw of the Lab. Chem. Pharmacology, NCI, PHS

Outside the PHS:
Dr. S. Burstein & Dr. R. I. Dorfman, Worcester Foundation Experimental Biology, Shrewsbury, Mass are actively collaborating on certain chemical aspects of the project at Shrewsbury. Dr. Burstein was a guest worker at the [illegible] laboratory for 10 working days in July 1954. Close consultation,

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not to my knowledge.

13. Cooperating Units etc. (cont.)

conferences and joint publications have been agreed on.

15. Jcd-511
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

a. 6 β-hydroxycortisole-a new steroid in human urine (Burstein, Nadel, Dorfman - Arch. Bioch. & Biophys.)
b. Tube separatory funnel for rapid extraction (Nadel and Highhouse - Chemist Analyst)
c. Compound E and glycogen deposition in the livers of scorbutic guinea pigs. (Nadel and Mulay - Federation Proc. - presented)
d. Morphology of adrenal gland and hydrocortisone metabolism in stressed and in tumor bearing guinea pigs. (Nadel, Burstein and Dorfman - Am. J. Clin. Path. - presented, Inter. Path. meeting)
e. Interrelationships of liver glycogen, deposition, corticosteroids and ascorbic acid in scorbutic guinea pigs (Mulay, Saslaw and Nadel, J. Clin. End.)
f. Isolation of excreted corticosteroids in the guinea pig in late scurvy (Burstein, Dorfman and Nadel, Federation - presented)
g. Non-parallel changes in cholesterol and ascorbic acid in the adrenal glands of malaria parasitized chicks (Taylor, Josephson, Greenberg and Nadel, J. Clin. Endocrin.)
h. An influence of age on the transplantation of leukemia from inbred to non-inbred guinea pigs. (Nadel, Fed. Proc.)
i. Acceptance of a thesis by L. L. Saslaw on work performed while he was a member of the Lab. of Path., and the awarding of the degree of M. S. to him by the Graduate Faculty of the Columbia College of George Washington University in June 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induction & Pathogenesis — SECTION OR SERVICE
4. Bethesda, Maryland — LOCATION (IF OTHER THAN BETHESDA)
5. ZC1-5/2 — SERIAL NO

"Effect of microbial and other agents on the development and course of neoplastic disease in animals." — PROJECT TITLE

J. M. Nadel (see #13 for additional information) — PRINCIPAL INVESTIGATOR(S)

(see #13 for additional information) — OTHER INVESTIGATORS

PROJECT DESCRIPTION

Present title: "Effect of microbial and other agents on the development and course of neoplastic disease in animals."

Previous: The present project title includes 3 sub-projects gathered under the project title of:

a) Mutual aspects of the pathophysiology of leukemia and malaria in animals. When it became obvious that the project on perfusion studies could not begin because of the administrative uncertainty of space, facilities and moving date to the Clinical Center laboratories, 2 other projects related to malaria were undertaken.

These projects include:

b) The effect of certain viruses on the course of cancer in animals and

c) The mode of action of certain living and chemical agents on the development and course of cancer in animals.

Objective: (1) To explore the activity of certain potentially carcinostatic and carcinogenic agents.

(2) To explore certain immunogenetic and endocrine aspects of resistance to malaria infection and to viruses helpful in a study of the carcinostatic potentiality of these microbial agents.

(3) The mode of action of certain virucidal and malariacidal drugs as carcinostatic agents.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

9. Project Description: (cont.)

Methods: The overall problem is studied by the combined used of techniques in pathology, microbiology, hematology, genetics, chemical pharmacology and immunology. Inbred, hybrid and specific backcross animals are studied for resistance to microorganisms such as the malaria parasite, neurotropic viruses etc. and the effect of these on the course of transplantable tumors is studied. Combinations of drugs and of viruses are studied.

Major findings:

(1) Guinea pigs with leukemia lived significantly longer after they were experimentally infected with the virus of lymphocytic choriomeningitis.(LCMV)

(2) Guinea pigs made immune to the LCM virus were afforded no protection when the virus was injected after transplantation of the tumor.

(3) Four drugs (ethionine, amethopterin and a purine and pyrimidine analogue) were studied to find that dose at which each individual drug was ineffective. Combination of these drugs at their individually ineffective dosages successfully prolonged the life of tumor bearing mice, more than 100% in comparison to controls.

(4) Repeated therapy with the combination of 4 drugs to tumor bearing mice resulted in the development of a resistant tumor. This resistant tumor is now being used as a means of studying the mode of action of other potentially carcinostatic drugs.

Significance to cancer research:

(1) A neurotropic virus (LCMV) made relatively benign by repeated passage to mice (300 generations) was able to prolong the life of leukemic guinea pigs from 19 to over 30 days. The virus did not of itself appear lethal to guinea pigs though other strains of the virus apparently are. This increase in survival is greater than that previously reported from this project, on the inhibitory effect of malarial infection on the course of leukemia in mice. This type of study has therapeutic implications for use in man.

(2) By judicious use of a tumor, resistant to four different types of chemotherapeutic agents, the mode of action of potentially carcinostatic drugs, with different chemical structure can be studied.

Proposed course:

Attempts to enhance the duration of the inhibitory effect of the microbial agents under study are being made. These include the judicious use of hormones, X ray irradiation, drugs and multiple sequential use of microbial agents. The possibility of the use of viral therapy in the form of the LCM virus for leukemia in man must be considered. Continued studies on the possible similarities in the genetics of resistance in mice to malaria and to leukemia, and the role of endocrines in such resistance are underway.

0. NCI-572
SERIAL NO.

1. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$8,173	1	1	2	½	2/3	1 1/6	0
FY 1956	$8,410	1	1	2	1/3	1	1 1/3	0

2. BUDGET ACTIVITY: Cooperating personnel assigned to the staffs of Dr. Greenberg, Chemotherapy Sec., Lab. Trop. Dis. NMI, Dr. Haas, Office of the Dir. NMI, Dr. Jay Lab. Aids Br., OD, NIH

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

3. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Not to my knowledge

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NCI-572
SERIAL NO.

5. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

"Effect of malaria on leukemia in mice"(Nadel, Greenberg, Coatney; J. Infectious Diseases)
Differences in survival of several inbred strains of mice and their hybrids infected with plasmodium berghei (Greenberg, Nadel, Coatney; J. Infect. Dis.)
Synergistic inhibitory action of amethopterin and a diaminopyrimidine on leukemia L 1210 in mice (Nadel, Greenberg; Cancer Research)
Increased resistance to malaria of certain inbred strains of mice, their hybrids and backcrosses (Nadel, Greenberg, Coatney and Jay; Am. J. Path.)

6. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

PROJECT REPORT FORM

1. National Cancer Institute	2.	Laboratory of Pathology		
INSTITUTE		LABORATORY OR BRANCH		
4. Cancer Induction & Pathogenesis		Bethesda, Maryland	5. ZC1-5713	
SECTION OR SERVICE		LOCATION (IF OTHER THAN BETHESDA)	SERIAL NO	

"Use of isolated organ perfusion techniques in a study on the pathologic physiology of tumor bearing animals."
PROJECT TITLE

E. M. Nadel
PRINCIPAL INVESTIGATOR(S)

None
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Project title: "Use of isolated organ perfusion techniques in a study on the pathologic physiology of tumor bearing animals."

Objective: Study changes in physiology accompanying tumor growth to learn how tumors divert energy sources away from the host, as a means towards the control of tumor growth.

Methods: The perfusion methods used have been used successfully in the hands of Dr. Leon Miller, while the micromethods to be used have been described by Drs. Scholander, Kirk and Natelson.

Major Findings: This project was begun in November 1953. There have been no findings inasmuch as work on this project has been delayed by unforseen administrative circumstances, including:

a. the transfer of an assistant to another laboratory,
b. inaccessibility of suitable laboratory space available in Building 8 and in the Warwick Clinic,
c. the move from Warwick clinic to Building 8,
d. the repeated indefiniteness of the time of the relocation of the laboratory in the Clinical Center.

Nevertheless some gains have been made including a consultation with Dr. Miller of Rochester on the techniques, the development of an incubated perfusion box after discussion with members of the Instrument Section, LAB, NIH, tooling up in terms of needed apparatus, and a review of the pertinent methods.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

9. Project Description (Cont.)

It is anticipated that little work can be done on this project until the principle investigator has what he considers satisfactory laboratory space and facilities for the project in a permanent laboratory. It is believed that such space and facilities may become available by June 1955.

NCI-513.
SERIAL no.

PROJECT REPORT FORM (Cont'd)

Ncl-513
SERIAL NO.

BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$1,755	1	1	2	-	1/3	1/3	0
FY 1956	$1,773	1	1	2	-	1/3	1/3	0

BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Cancer Induction & Pathogenesis — SECTION OR SERVICE
4. Bethesda, Maryland — LOCATION (IF OTHER THAN BETHESDA)

SMC/-514 — SERIAL NO

6. "Induction of tumors in Experimental animals and determining chemical changes in the host tissue concomitant with the growth of the neoplasm." — PROJECT TITLE

7. Dr. Ambadas S. Mulay — PRINCIPAL INVESTIGATOR(S)

8. Dr. H. L. Stewart, Dr. C. C. Congdon — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project title: "Induction of tumors in Experimental animals and determining chemical changes in the host tissue concomitant with the growth of the neoplasm."

Objectives: To evolve a technique which will induce desired tumor-like gastric carcinoma consistantly in high percentage of experimental animals.

To correlate the chemical changes in the tissues of the host with the growth of the tumor.

To study the chemistry of the tumor tissue, and the effect of tumor growth on the physiology of the host.

Methods employed: Feeding animals with various combinations of carcinogens and chemical and physical agents and observing the animals for development of tumors. Microscopic examination of the tissues for lesions.

Chemical analysis of the tissues of the host and of tumors for the changes brought about by the treatment and by neoplastic growth.

Major Findings: Increase of aldolase content of serum in animals with induced liver tumors. Differences in the aldolase content of arterial and venous blood, going in and out of liver bearing induced tumor. Differences in aldolase content of hepatoma, fibroma and sarcoma induced in rats.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Dr. Ambadas S. Mulay

A fast growing sarcoma was induced in Osborne-Mendel rats, at the site of injection, with 1 per cent solution of p-dimethylaminobenzene-1-azo-2-naphthalene, in polyethylene glycol.

Failure to induce gastric carcinoma in C57Br mice with combined treatment of gallium citrate injections and methylcholanthrene feeding.

Induction of forestomach tumors in C57BR mice which received 9.10-dimethyl-1,2-benzanthracene in triton by mouth.

Significance to Cancer Research: Possible evolution of chemical laboratory method which may help in early diagnosis and/or management of specific neoplasm like liver tumor.

Increase our knowledge of chemical carcinogenesis and tumor host relationships.

10. NC/-514
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$9928	½	1	1½	½	1	1½	-
FY 1956	$9956	½	1	1½	½	1	1½	-

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

not to my knowledge

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

National Cancer Institute	2.	Laboratory of Pathology	
INSTITUTE		LABORATORY OR BRANCH	
Cancer Induction & Pathogenesis	4.	Bethesda, Maryland	ZMCL-515
SECTION OR SERVICE		LOCATION (IF OTHER THAN BETHESDA)	SERIAL NO

"Influence of nutritional and hormonal factors on the incidence of spontaneous and induced neoplasms."
PROJECT TITLE

Shadas S. Mulay
PRINCIPAL INVESTIGATOR(S)

Dr. M. H. Bvestone, Dr. A. Symeonidis
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Project title: "Influence of nutritional and hormonal factors on the incidence of spontaneous and induced neoplasms."

Objective: To find nutritional and/or hormonal factor or factors, excess or lack of which will make an animal more susceptible to tumor induction.

Methods Employed: Feeding animals with high or low concentration of the factor or factors to be studied and observing the differences in the incidence of tumors in different groups. Microscopic examination of the tissues for lesions.

Influencing the hormonal changes in animals, with castration implantation or injection of hormones and observing the effect of these treatments on the incidence of cancer and other microscopic changes in the tissues of the experimental animals.

Influence of low protein (6 percent) Semi-Synthetic diet containing 0.06 per cent p-dimethylaminoazobenzene on the incidence of hepatoma and other lesions in the female Osborne-Mendel rats.

Influence of sex on the incidence of hepatoma in Osborne-Mendel rats fed 0.06 per cent p-dimethylaminoazobenzene in semisynthetic diet for 40 weeks.

Influence of methionine, choline, protein, riboflavin, and Vitamin B-12 concentration in the diet on the incidence of spontaneous liver tumors in C3Hf mice.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Dr. Ambadas S. Mulay

Chemical analysis of the tissues for quantitative changes in the factors under study. -

Major findings: In Osborne-Mendel rats fed semisynthetic diet containing 0.06 per cent p-dimethylaminoazobenzene, 90 per cent of the males develop hepatomas but only 25 per cent of the females develop hepatomas. (Percentages based on examination of over 100 animals of each sex.)

Female Osborne-Mendel rats fed on low protein (6 per cent casein) semisynthetic diet containing 0.06 per cent para-dimethylaminoazobenzene, develop hepatomas in only 10 per cent of the rats.

Adrenocortical carcinoma was observed in about 10 per cent (4 out 42) female rats, 18-30 months old, which had received, low protein diet containing 0.06 per cent para-dimethylaminoazobenzene for 40 weeks.

This adrenocortical carcinoma transplants readily and metastasizes to liver and lungs. This tumor shows some physiological activity, in as much as it depresses the growth of the adrenals of the host.

Adrenalectomised rats, or animals treated with desoxycorticosterone-acetate (atrophic adrenal glands) do not develop hepatic tumors when fed synthetic diet containing p-dimethylaminoazobenzene.

Not enough data on mice experiments to draw any conclusions.

Significance to Cancer Research: Knowledge of environmental factors influencing tumor growth may give us some clues in the management and control of cancer.

0. Ac1-515
SERIAL NO.

1. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$9,928	½	1	1½	½	1	1½	-
FY 1956	$9,956	½	1	1½	½	1	1½	-

2. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

3. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not to my knowledge

15. ZC1-515
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Effect of adrenalectomy and of desoxycorticosterone acetate on the formation of liver lesions in rats fed p-dimethylaminoazobenzene. A. Symeonidis, A. S. Mulay, and F. H. Burgoyne, J. Nat. Cancer Inst., 14:805-17; 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

National Cancer Institute	2.	Laboratory of Pathology	
INSTITUTE		LABORATORY OR BRANCH	
Office of the Chief	4.	Bethesda, Maryland	5 ZMcI-576
SECTION OR SERVICE		LOCATION (IF OTHER THAN BETHESDA)	SERIAL NO

"Induction, Pathogenesis and Morphological Alteration of Tumors Arising in Bone".
PROJECT TITLE

Dr. Albert W. Hilberg
PRINCIPAL INVESTIGATOR(S)

None
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Project title: "Induction, Pathogenesis and Morphological Alteration of Tumors Arising in Bone".

Objectives: The objective of this project is to develop a method of producing bone tumors in animals which are similar, morphologically, to those in man and to study the sequence in events which lead to tumor production or alteration in tumors already developed.

Methods employed: Experimental methods will vary with the difference in animal species, difference in tumor type, or difference in specific objective, i.e. study of pathologenesis or morphological alteration.

In general: rabbits, rats, mice and dogs have received injections of beryllium oxide by various routes such as intravenous, intraperitoneal, intramedullary, or introtracheal. At intervals, following injection, blood serum phosphatase determinations and skeletal surveys by X-ray examination have been done where applicable. All tissues from animals dying during the course of the experiments are examined grossly and representative tissue sections made for microscopic study. In addition X-ray examination is done on major bones and bones suspected of bearing tumor.

In the instance of study of alteration of bone tumors serial transplantation of spontaneously developing osseous tumors is carried out. Transplantation of tumor tissue into various tissue sites is done and after the tumor has grown to sufficient size of the tumor and site of transplantation are carefully examined by microscopic study of tissue sections. Studies are being conducted, primarily, of bone tumors transplanted to liver, kidney,

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

9. PROJECT DESCRIPTION (Cont.)

subcutaneous tissue and the peritoneal space.

Selective transplantation of an ovarian teratome (Fekete). This transplantable tumor in mice contains elements of all three germ layers. By selective transplantation, i.e. attempting to pick pure areas for transplantation, an effort is being made to separate the various germ layer elements of the tumor to see whether or not they will grow independently and whether or not each element is a malignant neoplasm by itself. The bone developed in this manner, does not appear to be malignant neoplastic tissue but it does grow in transplant.

Major findings: The experiments just described have not been carried to a final conclusion. To date a lymphoma and osteogenic sarcoma have developed in 2 rabbits injected with beryllium; a bone tumor has developed in a mouse injected with beryllium 12 months previously, and this tumor has been carried successfully in serial transplant.

The experiment having to do with morphological alteration of bone tumors is essentially completed. Only a few animals remain to be examined. The final report of the results is in preparation.

A survey of spontaneous bone tumors in mice has been completed to date. The preliminary report was given in an abstract entitled "Osteogenic Sarcoma of Mice" published in the Proceeding of American Association for Cancer Research, Vol. 1, April 1954, page 20; also in a talk given to the American Association for Cancer Research in April 1954. A final draft of the results, in more complete form, is in process.

Work with the ovarian teratoma has progressed to a point at which it is possible to obtain large areas of the tumor which will be composed of specific elements such as bone, squamous epithelium, and melanomatous tumor. Further separation of these elements is believed possible and the study of the malignant potentials of each element will then be possible.

Significance to Cancer Research:

Bone tumors are said to be among the three most common malignant neoplasms in children. These tumors develop at all ages and represent a major disaster to the patient since cures are extremely rare and treatment is usually mutilating. Attempts to learn more about the development of bone tumors, including contributing factors to development and the biological and morphological behavior after development, can give valuable information toward an ultimate goal of prevention, treatment or cure of these tumors. The information gained concerning specific substances, such as beryllium, which produce bone tumors, may be of value because of the industrial uses of beryllium and its compounds. This information may have direct implications to human tumor development.

9. PROJECT DESCRIPTION (Cont.)

Proposed course of project:

The continuation of the study of the animals injected with beryllium oxide in the manner prescribed will be of prime interest. The phases of the project concerned with alteration of the morphology of bone tumors will be completed and it is hoped reported in full. The reports of the spontaneous tumors will also be completed. New tumors, as they arise will be studied and added to the series.

Since the project director has increasing duties to the Pathologic Anatomy Branch as Head of the Cytodiagnosis Section, a gradual curtailment of research projects as listed, is anticipated. Collaboration in the beryllium studies with Dr. Steffee will continue as described by Dr. Steffee, and all studies presently in progress will be carried to a proper conclusion.

10. Rcl-516
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$7597	¼	1	1¼	¼	1	1¼	
FY 1956	$7636 less than	¼	1	1¼	~~less than~~ ¼	1	1¼	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No similar research is known to be in progress elsewhere.

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Laboratory of Pathology — LABORATORY OR BRANCH
3. Pathological Technology Section — SECTION OR SERVICE
4. Bethesda, Maryland. — LOCATION (IF OTHER THAN BETHESDA)
5. nc/-517 — SERIAL NO

Pathological Technology Section
PROJECT TITLE

Mr. J. M. Albrecht
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION

Stained tissue sections are the fundamental basis of all clinical and experimental studies of cancer. The section prepares histological sections for all the investigators of the National Cancer Institute. It makes available all the established routine and special stains and in addition develops and provides the current experimental methods of tissue preparation such as enzyme stains and specific histochemical stains.

January 1, 1954 - December 31, 1954

Number of Investigators	51
Number of Pieces	103,763
Number of Bottles	15,309
Number of Blocks	54,367
Blocks cut Serially	951
Frozen Blocks cut	604
Number of Autopsies	13,611
Number of Recuts	1,369
Number of Slides stained H & E	67,354

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION) (see next sheet)

9. PROJECT DESCRIPTION - continued

Number of Slides stained Special..... 12,043

Number of Slides Unstained..... 9,152

10. Hcl-517
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$137,245	3	23	26	3	20 3/4	23 3/4	0
FY 1956	$146,016	3	25	28	3	22	25	0

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. Metabolism Section — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NC(-600 — SERIAL NO

6. Mechanism of binding of p-dimethylaminoazobenzene to the liver in the production of hepatic tumors.
PROJECT TITLE

7. Julius White
PRINCIPAL INVESTIGATOR(S)

8. _____
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine the pathway of binding of p-dimethylaminoazobenzene to the liver protein, which ultimately leads to primary cancer of the liver in rats.

Methods Employed: The synthesis of N^{15} labeled intermediates of p-dimethylaminoazobenzene; the administration of these intermediates to rats; the determination of the utilization and the distribution of the intermediates; the isolation of the protein-bound compounds; the determination of the distribution of the N^{15}.

Major Findings: One of the unique properties of p-dimethylaminoazobenzene when fed to the rat is that tumors develop only in the liver and no other organ. Furthermore it has been shown that this carcinogen combines with some proteins of the liver to form a stable protein-dye complex. This remarkable specificity suggests that there is probably present in the liver, a protein which is characteristic for liver and probably not present in other organs. Hence the binding of the dye with the protein results in the impairment or progress in the normal utilization of this protein, leading to hepatic tumor formation. It is also noteworthy that the protein-bound dye continues to increase for approximately one month when a

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

maximum level is reached. Even though feeding of the dye is continued, there is a gradual disappearance of native dye. Experiments have shown that the binding of the protein occurs with one of the three nitrogens of the dye. In this experiment we have clearly demonstrated which of the three nitrogens are involved in this binding and have indicated that perhaps, the disappearance of the protein-bound dye is not in reality a disappearance but that part of the dye is destroyed during metabolism, leaving a fragment of the dye attached to the protein. We have suggested that the carcinogenic process proceeds in two steps: (1) The direct combination of the parent dye to the protein, and (2) The cleavage of part of the dye leaving the protein-bound to a phenylene diamine product. That perhaps it is this latter protein-bound phenylene diamine derivative is responsible for the carcinogenic process.

Significance to Cancer Research: To throw light on the mechanism involved in the formation of hepatic tumors as a result of feeding p-dimethyl-aminoazobenzene.

Proposed course of project: To present unequivocal evidence that the proposed mechanism is valid, it is proposed to synthesize p-dimethylaminoazobenzene with C^{14} in each of the two benzene rings and follow the fats of these compounds in the formation of the protein-bound dye complex and to actually determine which part of the dye is indirectly involved in the binding to the protein. Following this, isolation of the protein-bound dye and its characterization will be performed.

10. Ncl-600
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$11,886	1	2	3	1/3	1-2/3	2	
FY 1956	$ 4,865	1	1	2	1/3	1/3	2/3	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

15. NCI-600
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Julius White, Harriett T. Cool, Ruth S. Stander, and Philip W. David: Metabolism of N^{15} labeled p-dimethylphenylenediamine in rats. J. Nat. Cancer Inst. 15: 529-537, 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Metabolism Section (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-601 (SERIAL NO)

6. Relationship of protein intake and nitrogen excretion in rats following total body x-irradiation.
PROJECT TITLE

7. Julius White
PRINCIPAL INVESTIGATOR(S)

8. B. E. Burr
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine whether the level of protein intake influences the the effect of x-irradiation on the nitrogen excretion in the rat.

Methods Employed: Rats were fed one of three diets differing from each other only in the percent of casein and starch present. These diets were isocaloric and fed for at least 30 days prior to exposure to 450-500 r total body x-irradiation. Total nitrogen, urea and ammonia nitrogen were analyzed in the urine, and total nitrogen in the feces. Analysis of the food for nitrogen and record of food intake made it possible to determine the nitrogen balance.

Major Findings: Two major findings were made:

(1) Rats fed a low protein diet (6 percent casein) showed a marked increase in nitrogen excretion, while those ingesting a 15 or 20 percent casein diet showed a much lower increase in nitrogen excretion, after receiving 450-500 r total body x-irradiation. These increases in nitrogen excretion are, to a great extent, due to increased urea excretions and suggest increased protein catabolism. As a result of radiation exposure, loss of appetite ensues and, coupled with radiation effects, demands for energy from endogenous sources are increased.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

(2) Rats fed the low protein diet (6 percent casein) developed cirrhosis of the liver after 145 days in 53 percent of the animals which survived 450-500 r total body x-irradiation, while animals ingesting the 15 or 20 percent casein diet for periods as long as 450 days showed no cirrhosis.

Significance to Cancer Research: To determine whether exposure to x-irradiation can lead to cancer, studies as those described above have been designed. Atom bomb blasts have been shown to increase the incidence of leukemia in areas where radiation from the a-bomb appeared. The profound effect of x-irradiation exposure on the metabolism of the rat has been demonstrated with the resultant severe cirrhosis of the liver in animals on a relatively low protein diet. To date no evidence of hepatic tumors have been demonstrated.

Proposed course of project: To determine the biological factor or factors which cause this increased nitrogen excretion and methods of altering this effect; to determine if any other changes occur after radiation exposure.

10. NC1-601
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$25,123	1	3	4	1	3	4	
FY 1956	$ 9,956	1	1	2	1/2	1	1-1/2	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None.

15. NC/-60/
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Julius White, Bernard E. Burr, Harriett T. Cool, Philip W. David and Mona S. Ally. Level of protein intake and nitrogen excretion in rats following total body x-irradiation. J. Nat. Cancer Inst. 15: Feb. 1955.

Julius White, Charles C. Congdon, Philip W. David and Mona S. Ally. Cirrhosis of the liver in rats following total body x-irradiation. J. Nat. Cancer Inst. 15: Feb. 1955.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. Metabolism Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. MC-602 — SERIAL NO

6. The effects of x-irradiation on the nucleic acid and protein synthesis of the small bowel epithelium of the rat as related to cell division.
PROJECT TITLE

7. Julius White
PRINCIPAL INVESTIGATOR(S)

8. Dr. R. Bland Williams, Naval Medical Research Institute, Bethesda, Maryland
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objective of this project is to correlate histopathological changes in the small bowel epithelium produced by x-irradiation with nucleic acid and protein synthesis.

Methods Employed: Rats are exposed to total body x-irradiation of varying concentration (from threshold to lethal dose) and are sacrificed at varying times. The following histological changes are observed in the small bowel epithelium: (a) mitotic arrest, (b) acute cell destruction, (c) prolongation of interphase, (d) overshooting in recovery, (e) incomplete recovery. Parallel to these histopathological studies, the biochemical changes as characterized by the concentration of protein and nucleic acid are determined using N^{15} labeled glycine as the precursor in the synthesis.

Major Findings: It has been observed that with the arrest of cell division there was always associated an arrest in DNA (desoxyribosenucleic acid) synthesis but no depression in protein synthesis. If one examines the DNA synthesis zero to five hours after exposure to x-irradiation varying from 450 to 2,000 r there is an arrest of DNA synthesis. If however analyses are made on tissues 68-73 hours after x-irradiation there is a threefold increase in protein synthesis and an 8-fold increase in DNA synthesis. This increased synthesis has been associated with the cells which were in interphase and are now dividing. Exposure to

600 r results in increased synthesis after 31-36 hours; however, two exposures to 600 r, 48 hours apart, and synthesis studied 96-101 hours after the final exposure results in a 2-fold increase in protein synthesis while there is no increase in DNA synthesis. Exposure to 2,000 r results in a depression in DNA synthesis. If part of the bowel is shielded and the remainder of the bowel and animal are exposed to 2,000 r, the shielded loop shows no depression in protein and DNA synthesis 67-72 hours later even though the exposed part of the bowel shows complete depression.

Significance to Cancer Research: These data suggest a relationship between mitotic inhibition, small bowel injury and symptomatology. It is felt that this type of study may reveal that one can eventually destroy tumor tissue (in the small bowel) by knowing the effect of irradiation on cells in division, the time factor in recovery and the application of multiple doses of irradiation.

Proposed course of project: More data are needed on the correlation of changes in nucleic acid synthesis with the prolongation of interphase, effect of fractionating the dose of x-ray on the degree and duration of mitotic inhibition as indicated by DNA synthesis.

10. NC1-602
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$15,945	2	1	3	1-1/3	2/3	2	
FY 1956	$12,820	1	1	2	1	2/3	1-2/3	

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Naval Medical Research Institute, Bethesda, Maryland

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

No Entries for 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. Metabolism Section — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-603 — SERIAL NO.

6. The utilization of D and L forms of N^{15} labeled D and L-phenylalanine and valine for normal and tumor growth.
PROJECT TITLE

7. Julius White
PRINCIPAL INVESTIGATOR(S)

8. _____
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine the availability for protein and nucleic acid synthesis of normal and tumor tissue, labeled essential amino acids.

Methods Employed: Administration of the labeled amino acids to rats bearing transplanted Walker 256 carcinoma. Determining the uptake in protein and nucleic acid in the normal tumorous components.

Major Findings: To date analytical procedures have prevented any progress of a positive nature. We have established reliable procedures which involve column chromatography for the separation of the components. Columns and resins had to be designed which would handle the larger quantities of materials needed for this study.

Significance to Cancer Research: To study the growth requirements of tumor tissue and its relationship to ingredients available to both host and tumor.

Proposed course of project: To compare the availability and utilization of the essential amino acids for protein and nucleic synthesis by tumor tissue.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

10. NC1-623
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$6,620	1	1	2	1/3	2/3	1	
FY 1956	$9,728	1	1	2	2/3	2/3	1-1/3	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Radiation Branch
LABORATORY OR BRANCH

3. Metabolism Section
SECTION OR SERVICE

4.
LOCATION (IF OTHER THAN BETHESDA)

5. ZIC-6 [illegible]
SERIAL NO

6. The "Appetite Factor" in Walker Carcinoma 256
PROJECT TITLE

7. Julius White
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The observation was made by Dr. G. B. Mider that there is present in the Walker carcinoma 256 a factor which stimulates the appetite of tumor bearing animals. Animals bearing transplanted tumors reach a stage in growth whereby the tumor assumes massive proportions, at times approaching 50 per cent of the total weight of the animal. At this stage there is a marked loss in appetite and in weight. In the course of a few days the animal dies. However, if one adds as a supplement to an animal bearing a massive tumor at the time the appetite fails, a small amount of desiccated tumor tissue, there is an amazing stimulation of appetite and a resultant weight gain. This dramatic picture persists for as much as seven days when the animal succumbs as a result of direct or indirect presence of the tumor. There is therefore present in tumor tissue an "appetite and growth stimulating factor" which is not present in normal tissues investigated. The purpose of this project would be to study and isolate the active component of tumor tissue.

Methods Employed: The Walker 256 carcinoma would be grown in large quantities to supply material for adequate testing and purification.

Major Findings: To be determined.

Significance to the Cancer Research: A demonstration of the presence of an active factor present in tumor tissue which indirectly stimulates growth.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

10. NC1-604
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	0	--	--	--	--	--	--	
FY 1956	$ 4865	1	1	2	1/3	1/3	2/3	

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. Metabolism Section — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. ZC1-605 — SERIAL NO

6. Probable sources of available nitrogen for tumor growth. — PROJECT TITLE

7. Julius White — PRINCIPAL INVESTIGATOR(S)

8. _____ — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To study the availability of nitrogen for the rapidly growing small transplanted tumors. Does the tumor derive all of its nitrogen from the diet of the host or does the tumor impose a "stress" on stored tissues?

Methods Employed: Young growing animals will be placed on a semi-synthetic diet of known composition. At a given time, the Walker carcinoma will be implanted subcutaneously and at the same time, the animal will receive some N^{15} labeled glycine. This will be continued for about seven days or until such time when the tumor shows signs of growth. The N^{15} glycine will then be removed from the diet and at various stages during the growth of the tumor, determinations will be made to determine how much of the isotope has been removed from the host for tumor growth.

Major Findings: To be determined.

Significance to Cancer Research: To determine whether stored nitrogen can be built into cancerous tissue. Is there the phenomenon of the translocations of nitrogen from host to tumor.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

10. NC1-605
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	0	--	--	--	--	--	--	
FY 1956	$ 6,637	1	1	2	1/3	2/3	1	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. Metabolism Section — SECTION OR SERVICE
4. ____ — LOCATION (IF OTHER THAN BETHESDA)
5. ZMC-606 — SERIAL NO

6. Intermediary Metabolism of Histidine.
PROJECT TITLE

7. James C. Reid
PRINCIPAL INVESTIGATOR(S)

8. None.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: By comparative studies of the metabolism of histidine, a compound of basic importance to the economy of the animal, it is hoped to obtain information which will further our understanding of the basic nature of cancer. Histidine is of particular interest because it permits the study of both protein and nucleic acid synthesis from a single precursor.

Methods Employed: The tools being employed in the investigation are radioactive carbon and associated methodology.

Major Findings: The principal development during the year has been the emergence of a hypothesis that in Butter Yellow hepatoma of the rat there is a shift in the mechanism of nucleic acid synthesis. Normal rat liver utilizes small molecules extensively to build up nucleic acid purines; the tumor, on the other hand, appears to rely upon a supply of preformed purines. This hypothesis is based on the following facts: (1) Histidine functions as a nucleic acid precursor by producing formate, which is then used to build up purines; (2) incorporation of radiocarbon from labeled histidine into nucleic acid purines of normal rat liver is much greater than into those of rat hepatoma; (3) incorporation of labeled histidine into hepatoma protein is not reduced; (4) the nucleic acid content of the hepatoma is about the same as that of normal liver.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Significance to Cancer Research: Among the more effective therapeutic agents against cancer are the folic acid analogues, which inhibit condensation reactions of formate, and purine analogues, which inhibit synthesis of nucleotides from purines. Some cancers are vulnerable to one type of agent, others to the other. The development of resistance is commonly observed after an initial period of susceptibility.

If two biochemically important pathways of nucleic acid synthesis exist it is understandable that one tumor might respond to one type of blocking agent and another to the other, according to which pathway the tumor relies upon. Moreover, if a tumor can shift from one pathway to another, the development of resistance can be understood. These concepts are already generally recognized but good experimental verification is lacking.

Proposed course of project: During the next year it is proposed to carry out experiments to confirm the hypothesis outlined above. These will be of three types: (1) Administration of labeled adenine to show that incorporation into nucleic acids of Butter Yellow hepatoma is greater than in those of normal liver; (2) regression studies with folic acid analogues and purine analogues to show that the Butter Yellow hepatoma is inhibited more by the latter than the former; (3) metabolic studies during the development of resistance to purine analogues to demonstrate a shift in nucleic acid metabolism toward the utilization of formate. In this way it is hoped to put the questions discussed above on a firm experimental basis.

PROJECT REPORT FORM (Cont'd)

10. MCI-606
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 14,591	1	1	2	1	1	2	
FY 1956	$ 14,593	1	1	2	1	1	2	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None.

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Radiation Branch
LABORATORY OR BRANCH
Department of Biology,
Princeton University,

3. Office of the Chief
SECTION OR SERVICE

4. Princeton, N. J.
LOCATION (IF OTHER THAN BETHESDA)

5. ZC1-607
SERIAL NO

6. Fundamental effects of ultraviolet radiation on living systems, with regard to their relationships to the induction of cancer by that agent.
PROJECT TITLE

7. Harold F. Blum
PRINCIPAL INVESTIGATOR(S)

8. Prof. E. G. Butler and staff, Prof. A. K. Parpart, Dr. Murray Eden and Mr. John S. Cook - all of Princeton University. Prof. James Green of Rutgers University.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The principal aim of this project is a synthetic approach to the problem of carcinogenesis by ultraviolet radiation.

Methods Employed: The attack is a combined theoretical and experimental one. Using data available from our earlier work on the induction of cancer by ultraviolet light, a mathematical model has been constructed which explains certain aspects. Realizing that any such model can only have meaning if related to real biological phenomena, experimental studies of various effects of ultraviolet radiation on living systems have been undertaken. Our model would indicate that the mechanism of cell division and its control are of particular importance in this problem, and hence the effect of ultraviolet radiation on this phenomenon has been a principal preoccupation. Our studies have extended into related fields as this has seemed indicated.

Major Findings: During the present year, studies have continued in collaboration with Prof. E. G. Butler, and his staff, on effects of ultraviolet radiation on growth and regeneration in the urodele limb. It has been found that heavy doses of this agent are followed by extensive regression, and that in the ensuing regeneration process, supernumerary digits or limbs may appear, depending upon the area irradiated. Regression and

supernumerary growth are both diminished by following the ultraviolet radiation with "visible light".

Studies of the lytic action of ultraviolet radiation have been carried out by Mr. John S. Cook under my supervision. These have revealed unexpected quantitative relationships, and give some indication as to the mechanism, and possible bearing on our problem. (This work will be presented as Mr. Cook's thesis for the doctorate, and should be considered as confidential until a later time.)

Quantitative studies of polyspermia in the sea urchin's egg were carried on at the Marine Biological Laboratory, Woods Hole, in collaboration with Dr. Murray Eden. The data obtained are being analyzed.

Significance to Cancer Research: Perfection of aspects of our model of carcinogenesis has been attempted with certain limited success.

Proposed course of project: The studies of effects of ultraviolet radiation on amphibia will continue in collaboration with Prof. E. G. Butler. These will be extended to studies of the effects of ultraviolet radiation on cell proliferation and its control in mammalian tissues.

Studies of the lytic action of ultraviolet radiation will be continued, with particular regard to the question of photorecovery. Studies on photosensitized lysis and associated cation exchange will also be pursued, following preliminary studies already carried out. Aspects of the latter studies will be in collaboration with Professors A. K. Parpart and J. Green.

The analysis of data on polyspermia in the sea urchin's egg will continue, with the expectation of extension of the study with regard to abnormal cell division.

The synthetic approach to the problem of carcinogenesis by ultraviolet radiation will continue.

10. NC1-607
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 9325	1	0	1	1	0	1	
FY 1956	$ 9275	1	0	1	1	0	1	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Department of Biology, Princeton University

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

15. NC/-607
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Blum, H. F. and Kauzmann, E. F.: Photodynamic hemolysis at low temperature. J. Gen. Physiol. 37: 301-311, 1954.

Blum, H. F., Cook, J. S., and Loos, G. M.: A comparison of five effects of ultraviolet radiation on the Arbacia egg. J. Gen. Physiol. 37: 313-324, 1954.

Blum, H. F., Kauzmann, E. F., and Chapman, C. B.: Ultraviolet radiation and the mitotic cycle in the sea urchin's egg. J. Gen. Physiol. 37: 325-334, 1954.

Blum, H. F.: Photoorientation and the "Tropism Theory". Quart. Rev. Biol. December, 1954.

Blum, H. F.: Sunlight and Cancer. In Radiation Biology, vol. 2. A. Hollaender editor. New York, McGraw Hill. In press.

Blum, H. F.: Sunburn. In Radiation Biology, vol. 2. A. Hollaender editor. New York, McGraw Hill. In press.

Blum, H. F.: Physiological effects of sunlight on man. Meterological Monographs. In press.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Radiation Branch
LABORATORY OR BRANCH

3. Office of the Chief
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5 NC/-608
SERIAL NO

6. Bone Marrow Project
PROJECT TITLE

7. Charles C. Congdon, E. Lorenz
PRINCIPAL INVESTIGATOR(S)

8. _____
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Treatment of total-body irradiation injury. Determine the mechanism by which bone marrow and related living tissues prevent death from irradiation injury.

Methods Employed: Mice and guinea pigs exposed to lethal doses of total-body irradiation are injected with a variety of hematopoietic tissues from several species.

Major Findings: Rat bone marrow and bone was found to be effective in preventing death of irradiated mice. Mouse bone was also effective in irradiated mice. Guinea pig bone was effective in irradiated guinea pigs.

Significance to Cancer Research: These studies have led to new work in preventing irradiation induced leukemia and have allowed the use of massive doses of total-body irradiation in the treatment of transplanted leukemia.

Proposed course of project: Study of the mechanism by which the bone marrow and related tissues act.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

10. Ncj-608
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$29,438	1	5	6	1/3	5	5-1/3	
FY 1956	$29,632	1	5	6	1/3	5	5-1/3	

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM (Cont'd)

15. MC1-608
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Congdon, C. C., and Lorenz, E.: Humoral factor in radiation protection: Modification of lethal irradiation injury in mice by injection of rat bone marrow. Amer. J. Physiol. 176: 297-300, 1954.

Lorenz, E., and Congdon, C. C.: Radioactivity: Biologic effects of ionizing radiation. Ann. Rev. Med. 5: 323-338, 1954.

Lorenz, E., and Congdon, C. C.: Modification of lethal irradiation injury in mice by injection of homologous or heterologous bone. J. Nat. Cancer Inst. 14: 955-965, 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. Office of the Chief — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-609 — SERIAL NO

6. Radiation Effects Program.
PROJECT TITLE

7. Charles C. Congdon and E. Lorenz
PRINCIPAL INVESTIGATOR(S)

8. R. T. Haberman, F. P. Williams, J. White, J. Hollcroft
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Determination of acute and chronic total-body irradiation effects from the standpoints of life span, tumor incidence, hematology and general pathology.

Methods Employed: Mice, rats, and guinea pigs irradiated in a variety of ways were studied throughout life and by autopsy technique after death.

Major Findings: Bacterial infection in acutely irradiated mice was studied from the standpoint of general pathologic changes (Congdon, Williams, Haberman). Lesions in the intestinal tract were found which appeared to be one of the portals of entry.

In rats on a low protein diet, a dietary type of liver cirrhosis was observed following exposure to total-body irradiation (White, Congdon).

Tumor incidence and longevity in mice given chronic gamma irradiation of a very low intensity throughout their life span showed normal longevity and increased leukemia incidence in this experiment, confirming and extending an older piece of work (Lorenz, Hollcroft, Congdon).

Pathologic changes in the kidneys of mice given radon intravenously were observed and described (Hollcroft, Congdon). The lesions were of such a severity as to shorten the life of the mice.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Significance to Cancer Research: The importance of ionizing radiations in producing cancer, in treating cancer, and in studying the phenomenon of cancer requires a continual search for new radiation effects and an understanding of established ones. Treatment of irradiation effects requires intimate knowledge of the effects.

Proposed course of project: When new effects are encountered, preliminary study of their significance is undertaken to determine what role it might play in other projects.

10. HC-109
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 9693	1	2	3	1/3	1-1/4	1-7/12	
FY 1956	$ 9736	1	2	3	1/3	1-1/4	1-7/12	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Laboratory of Pathology and Histochemistry, NIAMD

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

15. NCI-609
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Lorenz, E., and Congdon, C. C.: Radioactivity: Biologic effects of ionizing radiation. Ann. Rev. Med. 5: 323-338, 1954.

Congdon, C. C., Williams, F. P., Haberman, R. T., and Lorenz, E.: The histopathology of bacterial infection in irradiated mice. J. Nat. Cancer Inst. In press, February, 1955.

White, J., Congdon, C. C., David, Philip W., and Ally, Mona: Cirrhosis of the liver in rats following total body x-irradiation. J. Nat. Cancer Inst. In press, February 1955.

Hollcroft, Joanne Weikel, Lorenz, Egon, Matthews, Marion, and Congdon, Charles C.: Long term survival following x-irradiation and the irradiation of the α particles from radon and its decay products. J. Nat. Cancer Inst. In press, February 1955.

Lorenz, Egon, Hollcroft, Joanne Weikel, Miller, Eliza, Congdon, Charles C., and Schweisthal, Robert: Long term effects of acute and chronic irradiation in mice. I. Survival and tumor incidence following chronic irradiation of 0.11 r per day. J. Nat. Cancer Inst. In press, February 1955.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. Office of the Chief — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. ZC1-610 — SERIAL NO.

6. Pathogenesis and Treatment of Leukemia.
PROJECT TITLE

7. C. C. Congdon, E. Lorenz, L. Law.
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Determination of the role of hematopoietic tissues in the pathogenesis and treatment of rodent leukemia.

Methods Employed: Cases of leukemia in guinea pigs that were irradiated or not irradiated were studied pathologically, as were the transplant lines established from them. Mice with a high incidence of leukemia were injected early in life with normal bone marrow from strains of mice that had either a low or a high spontaneous incidence of leukemia. Limited chronic gamma ray exposures were used to induce leukemia in guinea pigs. Transplanted leukemia in guinea pigs was treated by total-body irradiation alone or in combination with a chemotherapeutic agent.

Major Findings: Lymphatic leukemia occurs in guinea pigs as it does in other rodents. It was observed both in irradiated and non-irradiated guinea pigs (Congdon, Lorenz). Several transplant lines were established. The pathology followed that of other examples of rodent lymphatic leukemia.

Bone marrow from a strain of mouse with a high spontaneous incidence of leukemia increased the incidence of leukemia in its F_1 hybrid, while the injection of bone marrow from a low leukemic strain of mouse decreased the incidence of leukemia in its F_1 hybrid (Lorenz, Law, Congdon).

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Guinea pigs exposed to 8.8 r per day until they developed a marked anemia were removed from the exposure field. Subsequently, about 25 percent of the animals developed an acute aleukemic leukemia (Congdon, Lorenz). Bone marrow treatment of the anemia resulted in prompt return to normal values.

Massive doses of total-body irradiation cause prolonged remissions of transplanted guinea pig leukemia but cure was not obtained when the cumulative dose was 2,000 r. Bone marrow was used to keep the animals alive after the irradiation. Combination of small doses of x-rays with small doses of "tepa" did not show any greater effect than either agent alone when the treatments were administered nearly simultaneously (Lorenz, Congdon).

Significance to Cancer Research: Leukemia studies from the standpoints given here represent in part new and interesting approaches to this cancer problem. Further work is needed to determine their ultimate significance.

Proposed course of project: Repetition and extension of most of these experiments.

10. NCI-610
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$16,272	1	3	4	1/3	2-1/2	2-3/4	
FY 1956	$16,317	1	3	4	1/3	2-1/2	2-3/4	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Laboratory of Biology, NCI

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM (Cont'd)

15. NCI-610
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Congdon, C. C., and Lorenz, E.: Leukemia in guinea pigs. Amer. J. Path. 30: 337-359, 1954.

Lorenz, E., Law, Lloyd, and Congdon, Charles C.: The role of bone marrow and spleen in induced and spontaneous lymphatic leukemia. CIBA Foundation Symposium on Leukemia Research. Edited by G. E. W. Wolstenholme and M. P. Cameron. J. and A. Churchill, Ltd., London, 1954, pp. 189-195.

Lorenz, E., and Congdon, C. C.: Some aspects of the role of hematopoietic tissues in the pathogenesis and treatment of experimental leukemia. Proceedings of the Vth International Congress on Hematology, Paris, 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Radiation Branch
LABORATORY OR BRANCH

3. Office of the Chief
SECTION OR SERVICE

4. Donner Laboratory, University of California, Berkeley, Calif., and Radiation Branch, NCI, Bethesda
LOCATION (IF OTHER THAN BETHESDA)

SERIAL NO

6. Relative Biological Effectiveness Variation of Haploid Yeast.
PROJECT TITLE

7. Mortimer M. Elkind
PRINCIPAL INVESTIGATOR(S)

8. Dr. Carl A. Beam (Donner Laboratory)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objective of this research was to measure the biological effectiveness variation (alpha-particles compared to x-rays) of haploid yeast as a function of starvation.

Methods Employed: Survival curves were obtained for alpha-particle and x-ray irradiation as a function of the metabolic state of the cell. From a comparison of such survival curves, the relative biological effectiveness (RBE) was obtained. The range of metabolic states examined was from rapidly dividing log phase cells, to cells starved up to 9 days in a buffered dextrose medium (no fixed nitrogen source).

Major Findings: It was found that starvation caused the RBE to vary by a factor of 3 mainly as a result of a variation in the x-ray sensitivity. That is, alpha-particles were less effective than x-rays in killing rapidly-dividing, interdivisional cells while the reverse was true for starved interdivisional cells. This illustrates that the metabolic state has as profound an effect on the RBE of this system as the purely physical parameters previously thought to be responsible for RBE variation.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Significance to Cancer Research: While all new biological results can be ultimately of significance to cancer research, this data is pertinent for the following reasons: First, sites sensitive to radiation have been shown to be dependent on the physiological state of the system. Secondly, current theories of the basic mode of interaction of radiation with living matter must be re-examined since effects such as these are not predicted. Thirdly, the observed correlation between biological and physical parameters suggests that experiments of this type can aid in elucidating biological processes.

Proposed course of project: When a soft x-ray machine and alpha-particle source become available for this research, it is planned to investigate: (A) the relative effects of more diffuse forms of radiation as a function of starvation; (B) the relationship between ploidy and starvation; and (C), as time and assistance permits, the dependence of radiosensitivity on the composition of the growth medium.

PROJECT REPORT FORM (Cont'd)

10. RC/-611
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$9325	1	0	1	1	0	1	
FY 1956	$14,523	1	1	2	1	1	2	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

1955--Donner Laboratory, University of California, Berkeley, California.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None.

PROJECT REPORT FORM (Cont'd)

15. 2-41
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

Delivered an invited Biophysics Seminar at the Sloan-Kettering Institute for Cancer Research, New York City, on September 30, 1954, entitled "Radiosensitivity and Relative Biological Effectiveness Variation of Haploid Saccharomyces Cerevisiae."

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Office of the Chief (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. ZIC/-6/2 (SERIAL NO)

6. Comparison of Biologic Effects of α rays from Radon with X-rays.
PROJECT TITLE

7. Joanne Hollcroft
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine the difference in effect of X-rays with low specific ionization from those of α rays with high specific ionization.

Methods Employed: Study of long term survival of mice receiving equivalent energy doses of α irradiation and x-irradiation and investigation of kidney damage produced by these radiations. Study of lung tumor induction by α rays and x-rays.

Major Findings: (1) Life span of strain A male mice was shortened by 50% by α rays from radon and its daughters while it was not shortened by an equivalent dose of x-rays. Histologic studies showed that the α particles caused an early damage to the kidney from which the animals recovered and a severe later damage in which bizzare nuclei was seen and which accounted for the early death of the animals. The radon and its decay products had been found to concentrate in the kidney and thereby producing a kidney dose 14 times the average dose. Equivalent x-irradiation dose (14 x 400 r) to the kidney alone also produced an early damage from which the mice appeared to recover and later atrophy, calcification

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

of tubules and thickening of glomeruli. (2) Histologic studies confirmed the gross observations that neither an acute dose of x-irradiation nor a single dose of radon increased the incidence of lung tumors in Strain A mice.

Significance to Cancer Research: Comparison of biologic effectiveness gives insight into mechanism of radiation damage and is related to the radiotherapy of cancer.

Proposed course of project: A brief study on induction of kidney tumors by radon is in progress but no further studies are planned.

10. MCI-612
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$1755	1	1	2	--	1/3	1/3	
FY 1956	0	--	--	--	--	--	--	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

15. NYO-612
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Hollcroft, J. W., Lorenz, E., Congdon, C. C. and Matthews: M.: Long term survival following x-irradiation and the irradiation of the α particles from radon and its decay products. J. Nat. Cancer Inst. In press.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. Office of the Chief — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)

SMCI-613(a) — SERIAL NO

6. X-radiation treatment of a transplanted, metastisizing lymphosarcoma, L2.
PROJECT TITLE

7. Joanne Hollcroft
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine the lethal x-radiation dose for the L2 tumor.

Methods Employed: Strain A mice bearing L2 lymphosarcoma were irradiated starting on the 10th day after transplant. A bioassay of the liver, spleen and tumor from these mice was performed by inoculating strain A mice subcutaneously with these tissues at various times following treatment.

Major Findings: No tumor grew from implants of spleen or liver from mice irradiated with a single dose of 2,000 r or 2 daily doses of 1,000 r. Implants grew in all animals transplanted with tumor from mice irradiated 2, 3 or 4 days previously but not in all mice transplanted with tumor from mice irradiated 1 day previously. Implants of liver, spleen and tumor from non-irradiated mice all grew equally well.

Significance to Cancer Research: Radiotherapy of leukemia.

Proposed course of project: Continuation of this study using further fractionation and higher doses.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

10. MCI-613(a)
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$4,463	1	2	3	1/3	1/3	2/3	
FY 1956	$4,465	1	2	3	1/3	1/3	2/3	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Radiation Branch
LABORATORY OR BRANCH

3. Office of the Chief
SECTION OR SERVICE

4.
LOCATION (IF OTHER THAN BETHESDA)

5. ZC-013(B)
SERIAL NO

6. Modification of Radiation Effects on a Transplanted Lymphosarcoma.
PROJECT TITLE

7. Joanne Hollcroft
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine in what manner the tumor reacts to ionizing radiation and in what manner the radiosensitivity may be increased.

Methods Employed: Vary the physiology of the host (Strain A mouse) prior to, during, or following irradiation of the transplanted lymphosarcoma, L1; follow tumor growth.

Major Findings: (1) Most of the work on this project has been an effort to produce a more uniform tumor response to irradiation. When tumors were treated with 2000 r or 1000 r to the tumor plus 50 r to the body no difference in variation of response was seen whether the tumors were treated at 12, 15 or 18 days following transplant. Similarly no difference in response or variation of response was seen when small tumors were compared with large tumors treated at the same time following transplant. (2) A treatment of 1000 r to the tumor plus 50 r to the body daily for 3 days was more effective than a treatment of 1000 r to the tumor daily for 3 days.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

(3) Repeating treatment 1000 r to the tumor plus 50 r to the body when the tumors return to original size, in general, eventually resulted in radioresistance of the tumor but some tumors responded better after the second or third treatment than after the first treatment. (4) Treating the tumors with 1000 r to the tumor plus 50 r to the body while the animals were breathing 95% O_2 and 5% CO_2 appeared to be more effective than the same irradiation when the mice were breathing air. (5) Diathermy prior to irradiation did not alter the response. (6) Histamine injection immediately prior to irradiation decreased the tumor response following 1300 r to the tumor or 400 r whole body.

Significance to Cancer Research: To find methods of increasing usefulness of radiotherapy in cancer.

Proposed course of project: It will be attempted to change the radiosensitivity of L1 and other tumors by changing vascular supply, giving metabolic poisons, hormones and other drugs. Further studies on dose fractionation will be carried out.

10. MCI-613(6)
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 8,997	1	2	3	1/3	1	1-1/3	
FY 1956	$10,183	1	2	3	1/3	1-1/3	1-2/3	

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None.

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None.

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Office of the Chief (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5 ZMC-614 (SERIAL NO)

6. Long Term Survival and Tumor Incidence Following Acute or Chronic Irradiation. (PROJECT TITLE)

7. Eliza Miller (PRINCIPAL INVESTIGATOR(S))

8. Joanne Hollcroft and Charles C. Congdon (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives: To study long term survival and carcinogenesis of: (1) Chronic irradiation in the tolerance range. (2) Chronic irradiation in castrated female mice (mainly mammary tumor induction). (3) Acute radiation doses when the animals are protected from the acute irradiation effects.

Methods Employed: (1) LAF_1 mice were exposed to 0.11 r per 8 hour day γ irradiation for duration of life. (2) C_3H_f female mice were castrated and exposed to 8.8 r per 8 hour day. Life span and tumor incidence were compared with intact C_3H_f female mice receiving the same irradiation treatment as well as with intact and castrated controls. (3) C_3H mice were x-irradiated under the following conditions: (a) 400 r at birth. (b) 400 r with sham spleen shielding. (c) 400 r with spleen shielding. (d) 900 r with spleen shielding. (e) 900 r with chemical protection of 950 mg per kg cysteine prior and 95% He plus 5% O_2 during irradiation.

Major Findings: (1) Male LAF_1 mice exposed to 0.11 r per 8 hour day lived longer than the control LAF_1 male mice. Irradiated mice showed a higher "leukemia" incidence and a higher mammary carcinoma incidence than the control mice. The irradiated females also had a higher ovarian tumor incidence. (2) No difference in mean survival time was found between C_3H_f intact females and C_3H_f castrate females.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Chronic exposure to 8.8 r per day decreased the life span of both intact and castrate mice by the same amount. (3) The survival time following acute exposures was proportional to the dose and not influenced by the spleen protection, cysteine plus anoxia protection or irradiation at birth. Histologic findings on the mice irradiated at birth indicated an increase in ovarian tumors and adrenal tumors. These mice developed kidney cysts and had a high incidence of auricular and valvular thromboses. The mammary glands of these mice were undeveloped.

Significance to Cancer Research: Irradiation is a good tool for production of cancer. The lowest chronic dose which will produce certain tumors must be established. The long-term effects of acute doses of total body irradiation are of consequence in considering the role of radiotherapy in treatment of cancer.

Proposed course of project: Completion of histologic studies.

10. NCI-614
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$ 6,620	1	1	2	1/3	2/3	1	
FY 1956	$ 6,637	1	1	2	1/3	2/3	1	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM (Cont'd)

15. Mc1-614
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Lorenz, E., Hollcroft, J. W., Miller, E., Congdon, C. C. and Schweisthal, R.: Long term effects of acute and chronic irradiation in mice. I. Survival and tumor incidence following chronic irradiation of 0.11 r per day. J. Nat. Cancer Inst. In press.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. Metabolism Section — SECTION OR SERVICE
4. ____ — LOCATION (IF OTHER THAN BETHESDA)
5. ZIA CL-06154-(a) — SERIAL NO

6. Iodide Concentrating Mechanism in the Thyroid Gland. — PROJECT TITLE

7. S. H. Wollman — PRINCIPAL INVESTIGATOR(S)

8. Dr. R. O. Scow of NIAMD. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To learn about the mechanism by which the thyroid gland maintains a concentration of radioiodide elevated above that in the blood and about the way the thyroid radioiodide and iodide concentrations influence the uptake of radioiodine.

Methods Employed: Kinetic studies will be made of the ratio of radioiodide concentrations in the thyroid gland and serum and factors which control it.

Major Findings: The ratio of the radioiodide concentrations in thyroid gland and serum (T/S ratio) was independent of the serum iodide concentration at low concentrations in mice in which binding of iodine was blocked by propylthiouracil. At high enough serum iodide concentrations the T/S ratio decreased progressively as the serum iodide concentration increased. The observed data can be fit quantitatively by two qualitatively different models for the iodide concentrating mechanism, an active transport model and an adsorption model. In both models the T/S ratio decreases with increasing serum iodide concentrations because of a saturation of the iodide concentrating mechanism. In both models the iodide complexes with a substance in the thyroid, and the affinity of this substance for iodide is found to be the same for normal and hypophysectomized mice.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

The T/S ratio was measured in mice using graded doses of propylthiouracil and of iodide to block binding to various degrees. As the binding is progressively blocked the T/S ratio increases for both agents. However, after binding is almost completely blocked, a further increase in propylthiouracil dose causes no further change in the T/S ratio until toxic doses of goitrogen are reached, whereas with iodide a further increase causes a drop in the T/S ratio because of saturation of the iodide concentrating mechanism.

When binding was blocked with propylthiouracil and iodide the T/S ratio was higher than when the binding was blocked by iodide alone. This demonstrates that in at least one circumstance propythiouracil can elevate the T/S ratio without changing the extent of binding.

The T/S ratio was measured in normal and hypophysectomized rats. Blocking binding of radioiodine with propylthiouracil was associated with an elevation of the T/S ratio. The effects are similar but more striking than those in the mouse.

Studies of the amount of stable iodine bound as radioiodide binding is blocked by high concentrations of stable thyroid iodide suggests that two mechanisms exist for the blocking of binding. At lower levels of stable thyroid iodide the decrease in radioiodine binding appears to be a saturation of the binding mechanism, but at high enough levels the effect is due to an inhibition of the binding mechanism.

Significance to Cancer Research: Transplantable tumors of the thyroid gland in mice appear to have a marked impairment in or loss of the iodide concentrating mechanism. This loss appears directly responsible for the loss of ability of certain lines of thyroid tumors to accumulate radioiodine. The above experimental results are directed largely to provide an understanding of the iodide concentrating mechanism and the factors controlling it.

Proposed course of project: Further kinetic studies on the iodide concentrating mechanism will be undertaken. In addition, a more direct attack will be made on the problem of determining the factors which most seriously limit the accumulation of radioiodine.

10. VC/-6.5(a)
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$7,500	1	1	2	1/3	1/3	2/3	
FY 1956	$4,500	1	1	2	1/3	1/3	2/3	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

NIAMD, Dr. R. O. Scow.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

15. NC/-615(a)
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

S. H. Wollman: A thyroid model describing kinetics of exchange, concentrating, and organic binding of iodide. Endocrinology 54: 35-47, 1954.

S. H. Wollman and R. O. Scow: Dependence of ratio of radioiodide concentrations in thyroid gland and serum on serum iodide concentration: With propylthiouracil. Endocrinology 55: 828-836, 1954.

S. H. Wollman and R. O. Scow: Dependence of ratio of radioiodide concentrations in thyroid gland and serum on serum iodide concentration: Without propylthiouracil. Endocrinology 55: 837-844, 1954.

S. H. Wollman and R. O. Scow: Effect of various goitrogens and of dose of propylthiouracil on the ratio of radioiodide concentrations in the thyroid gland and serum in mice. Endocrinology. In press. Probable date of appearance March 1955.

S. H. Wollman and R. O. Scow: Effect of propylthiouracil on the ratio of the radioiodide concentrations in thyroid gland and serum in normal and hypophysectomized rats. Endocrinology. In press. Probable date of appearance March 1955.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None.

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Radiation Branch
LABORATORY OR BRANCH

3. Metabolism Section
SECTION OR SERVICE

4. .
LOCATION (IF OTHER THAN BETHESDA)

5/NCI-615(L)
SERIAL NO

6. Transplantable Thyroid Tumors: Production and I^{131} Metabolism.
PROJECT TITLE

7. S. H. Wollman
PRINCIPAL INVESTIGATOR(S)

8. Dr. R. O. Scow of NIAMD.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

(a) To produce transplantable thyroid tumors in the rat and the mouse.

(b) To compare iodine metabolism in thyroid tumors and normal thyroid.

Methods Employed:

(a) Thyroid tumors are being produced by chronic feeding of thiouracil.

(b) Iodine metabolism was studied with the use of radioactive iodine.

Major Findings:

(a) Transplantable thyroid tumors have been produced in rats and mice. The first thyroid tumors which will grow in our strain of rats fed a goitrogen-free diet were obtained this year. The mouse tumors still will grow only in hosts fed thiouracil.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

(b) Although no radioiodine studies have been made on tumors this year a study has been made of the effect of transplanting a normal thyroid lobe to a subcutaneous site. It was found that when the auto-transplanted lobe was compared with the lobe in situ there was no difference in growth in response to propylthiouracil feeding and no appreciable difference in the blood radioiodine clearance per unit weight of tissue in either normal, hypophysectomized or propyl-thiouracil-fed rats.

Significance to Cancer Research: The observed equivalence of autotransplanted thyroid tissue and tissue in situ suggests that the observed decrease in blood radioiodine clearance by transplanted thyroid tumors is due to biochemical changes in the tumor and is not due to the abnormal site. From the standpoint of radioiodine therapy it also suggests that metastasized tumors should not be more difficult to get radioiodine into just because they happen to be at an abnormal site.

Proposed course of project: Studies of the radioiodine metabolism of thyroid tumors in the rat will be undertaken this year.

10. ZC1-6/5 (B)
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$4863	1	1	2	1/3	1/3	2/3	
FY 1956	$4,865	1	1	2	1/3	1/3	2/3	

12. BUDGET ACTIVITY:

RESEARCH ☑ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

NIAMD personnel, Dr. R. O. Scow.

4, IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project may resemble research in the Laboratory of Biochemistry.

PROJECT REPORT FORM (Cont'd)

15. NCI-6154
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

S. H. Wollman and R. O. Scow: Comparison of thyroid lobes, auto-transplanted and in situ, in the rat: Growth and I^{131} uptake. J. Nat. Cancer Inst. In press. Probable date of appearance Feb. 1955.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Metabolism Section (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. ZIA CL-616 (SERIAL NO)

6. Autoradiographic Localization of I^{131} in Normal and Neoplastic Thyroid Tissue.
PROJECT TITLE

7. S. H. Wollman
PRINCIPAL INVESTIGATOR(S)

8. Mr. I. Wodinsky, Laboratory of Chemical Pharmacology, NCI
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

(a) To locate the sites of high concentration of radioiodide in the thyroid gland.

(b) To locate the site at which the binding of radioiodine occurs in the thyroid gland, and to determine changes in the localization of the I^{131} after binding.

Methods Employed: High resolution autoradiography.

Major Findings:

(a) No new results this year.

(b) Protein-bound I^{131} (PBI^{131}) first appears in the thyroid in the colloid at less than 11 seconds after injection of radioiodide. The PBI^{131} is at first localized to the periphery but in the course of 10 or 15 minutes becomes uniformly mixed in most of the follicles. The rate of mixing

varies from follicle to follicle, and in a small per cent of the follicles it is not uniformly mixed even after one hour. Nembutal anaesthesia prior to radioiodide injection markedly inhibits the mixing process. In the mouse some follicles are non-functional and do not bind I^{131}. On the other hand in the rat all follicles appear to bind I^{131} all the time.

Significance to Cancer Research: The appearance of PBI^{131} in the colloid and not in the cell offers an explanation for why thyroid tumors which do not have colloid do not accumulate I^{131}.

Proposed course of Project: Change in emphasis to localization of radiodide.

10. Z01-616
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$7305	2	1	3	1/3	1/3	2/3	
FY 1956	$7805	2	1	3	1/3	1/3	2/3	

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Laboratory of Chemical Pharmacology

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

15. NC1-616
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

S. H. Wollman and I. Wodinsky: Localization of protein-bound I^{131} in the the thyroid gland of the mouse. Endocrinology: In press. Probable date of appearance, January 1955.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. Physical Chemistry Section — SECTION OR SERVICE
4. ____ — LOCATION (IF OTHER THAN BETHESDA)
5. ZMCI-617 — SERIAL NO.

6. In Vivo Measurement of Tumor pH with the Capillary Glass Electrode.
PROJECT TITLE

7. H. Kahler
PRINCIPAL INVESTIGATOR(S)

8. Mrs. B. Haines
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objective of this project is to determine the in vivo pH of tumors under different experimental conditions.

Methods Employed: Following anesthesia, 3 fine tipped capillary electrodes were inserted into the tumor of each rat, 3 rats being investigated simultaneously, and the pH was recorded by automatic equipment over a period of 18 hours. After an initial steady state had been attained an injection of one of the numerous sugars was usually made.

Thermocouples have been used in the latter part of the year to record tumor and body temperatures. A heating device has also been installed to hold the rats' body temperature more nearly constant during the period of observations.

Major Findings: Maintenance of the body temperature to nearly the normal value has but a slight effect upon the pH drop of tumor on glucose injection but it speeds up the recovery to normal value following the drop in pH.

A slight drop in pH of the normal muscle following sugar injection has been consistently noted. This drop is not specific to glucose. Following the drop the recovery is also speeded up by warming the animal.

It has been found that more divergent pH values are obtained near the periphery of a tumor than at the central region.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Significance to Cancer Research: The pH of a tissue is one of its basic properties, and where this pH deviates from the normal, as in a tumor, the detailed study of these changes are essential to an understanding of tumor chemical processes.

Proposed course of project: The continuation of this study requires the measurement of large numbers of animals to make any conclusion statistically significant, particularly conclusions concerning small effects. It is planned to continue measurements directed towards determining pH gradients from the central core of the tumor (often necrotic) to the normal zone outside the tumor. More information is also needed concerning the nature of the small pH drop of normal tissue following sugar injection.

10. Ncj-617
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$9,728	1	1	2	1/2	1	1-1/2	
FY 1956	$9,956	1	1	2	1/2	1	1-1/2	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

15. NCI-617
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

H. Kahler, W. R. Bryan, B. J. Lloyd, Jr. and J. B. Moloney:
The density of the Rous sarcoma virus in sucrose solutions.
J. Nat. Cancer Inst. 15: 331-336, 1954.

H. Kahler, W. R. Bryan, B. J. Lloyd, Jr., and J. B. Moloney:
The sedimentation of the Rous sarcoma virus. J. Nat. Cancer Inst. 15: 337-339, 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. Physical Chemistry Section — SECTION OR SERVICE
4. ____ — LOCATION (IF OTHER THAN BETHESDA)
5. ZIA CI-CI8 — SERIAL NO.

6. Physical-Chemical Characterization and Comparison of Nucleic Acids, Nucleoproteins and Nucleases of Normal and Neoplastic Tissues. — PROJECT TITLE

7. Dr. Joseph Shack — PRINCIPAL INVESTIGATOR(S)

8. ____ — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objective of this project is to isolate, fractionate, characterize and compare nucleic acids and nucleoproteins of various normal and malignant tissues.

Methods Employed: Nucleic acids and nucleoproteins are isolated from tissues by a variety of mild methods and their properties are investigated by numerous procedures which have included ultraviolet spectrophotometry, electrophoresis, high speed centrifugation, electrometric titration, viscosimetry, and determination of susceptibility to degradation by acid, alkali, heat and specific enzymes.

Major Findings: A substantial portion of the calendar year was occupied in closing the activity in San Francisco, moving to Bethesda and setting up a laboratory here. Results during this year include the development of micro-methods for investigating the changes that occur on mild denaturation of nucleic acid and nucleoproteins by acid, alkali or heat and the application of the methods to the desoxypentose nucleic acids of mouse lymphoma and calf thymus. The findings support the concept that the desoxypentose nucleic acid of a single tissue consists of a heterogeneous mixture of related substances which differ in their susceptibility to denaturation.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Significance to Cancer Research: The significance of this project to cancer research lies in the contributions it may make: 1) To an understanding of the nature and properties of the nucleic acids and nucleoproteins of malignant tissues, and 2) To determining whether and if so, in what respects, these important components of the cell may differ among various normal and malignant tissues. It is to be expected that the isolation and characterization of the nucleic acid components will be useful in further studies of their metabolism, and of their interaction with such agents as radiation, carcinogens and chemotherapeutic compounds.

Proposed course of project: The proposed course of this project for the next year mainly involves an extension of what has been outlined above along the following lines: a) Isolation and fractionation of nucleic acids and nucleoproteins from tumors and normal control tissues; b) further development of physico chemical methods of characterizing these substances; c) further studies of the depolymerizing enzymes of these tissues and d) use of such physico chemical methods and of the specific enzymes to characterize these nucleic acids and nucleoproteins.

10. Mct-618
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$13,987	2	--	2	1-1/2	--	1-1/2	
FY 1956	$14,593	1	1	2	1	1	2	

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. Radiation Physiology Section — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)

ZRC1-619 — SERIAL NO

6. Energy Metabolism
PROJECT TITLE

7. A. W. Pratt
PRINCIPAL INVESTIGATOR(S)

8. W. C. White and M. Sultzer
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The overall objective of the program is to accomplish a continuous, accurate measure of the energy exchanges of experimental animals. The immediate objective for the past three years has been the construction of new instruments which will give an accurate, continuous measure of the gaseous exchanges of animals under study.

Methods Employed: Not applicable.

Major Findings: During the two previous years our research development program had led to the construction of two pilot instruments which were considered satisfactory for application in the study of energy metabolism of experimental animals and possibly human subjects.

The first of these instruments had been designed solely for use with small experimental animals. The instrument was in routine use approximately 160 days during the first 6 months of the year in the study of the energy metabolism of x-irradiated rats. During this period only three rats were studied since only one animal could be studied at a time. The data obtained on this small group will not be listed here except to say that accurate energy balance could be obtained over prolonged experimental periods and that basically the design of the instrument was satisfactory. The routine use of the instrument during this period pointed up both major and minor objections which limited the value of the instrument. It was obvious that it would take a very long time to accumulate a volume of data and any expansion of the program would necessitate complete duplication of all components. Thus, to obtain adequate

facilities would mean high costs. A further major objection stemmed from the amount of personnel time necessary for the routine processing of the data being obtained. Any expansion of the program would demand too much in the way of extra personnel. Discussions with the Chief of the Radiation Branch and the Scientific Director led to the decision to incorporate some of the newer developments in computer instrumentation into the recording system. Accordingly an analog to digital converter unit (Teleducer) was purchased. This unit can measure small D. C. voltages accurately and converts these signals into digital equivalents which are in turn tabulated into an automatic electric typewriter. With this device the processing of routine data is reduced to two operations saving considerable personnel time. The Teleducer has 50 measuring channels thus affording a potential animal load of ten animals using 5 channels per animal. Time intervals between readings however will reduce the maximum number of animals which can be studied to five animals.

Because of electrical interactions between the Teleducer and the power supply of the sensing elements there has been a delay in completing the new instrument. The high sensitivity of the Teleducer places stringent requirements on electric noise level of the power supply. We have determined that conventional regulated D.C. power supplys are not satisfactory in this application and that a development program will be needed to put the instrument on regulated line operation. It was decided that this development study would be deferred and the instrument operated by a battery supply. The battery supply is now in use and appears to be quite satisfactory and probably can be continued indefinitely. The initial standardization and calibration studies which have just been completed indicate that this instrument will be accurate and reproducible. It is expected that the instrument will be inroutine operation in January 1955.

The second instrument, originally intended for clinical use, consisted solely of a coupled analyzer and recorder assembly. This instrument was initially designed as a compromise unit in that analyzer-recorder assembly could be used with either human subjects or experimental animals by use of the necessary flow and sampling system.

Because of the many technical problems which are certain to arise in any human subject application it was decided that the initial use of the instrument should be with experimental animals. In this way the basic design could be critically tested and pertinent performance data could be obtained. According, the instrument has been constructed as a self contained, portable machine. An intensive calibration program has been finished which has uncovered a few minor errors in design which we believe have now have been corrected. There seems little doubt that this instrument is satisfactory for use in the physiological studies for which it was designed. The instrument is now being put in routine use.

During this year a second analyzer-recorder assembly has been received and this is now being assembled into an animal instrument. It is estimated that calibration of the instrument will begin in January.

It should also be noted that the two "clinical" machines can be converted whenever necessary for clinical use.

A major problem which we believed solved, that has delayed the program, is that of calibration of the instruments in terms of an absolute scale. To this end we constructed an alcohol lamp which coupled with mass spectrometric analytical techniques afford a rather accurate basis for calibration.

Significance to Cancer Research: By these measurements it is hoped that the energy balance aspects of x-irradiated animals and tumor-bearing animals can be accurately quantitated.

It is felt that such knowledge would add in considerable measure to the physiological and chemical concepts of the nature of physiological injury and tumor growth.

Proposed course of project: We believe that the initial phase of this development program has been satisfactorily completed. It is now planned that the instruments will be put in routine use for data collection. It is also contemplated that the second phase of the development program will now get underway, namely that of building direct calorimeters.

10. MC1-619
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$13,239	2	2	4	2/3	1-1/3	2	
FY 1956	$18,592	2	3	5	2/3	2-1/3	3	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Physiology Section (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-620 (SERIAL NO)

6. Study of the Radiochemical Decomposition of β-OH Aliphatic Organic Acids.
PROJECT TITLE

7. A. W. Pratt
PRINCIPAL INVESTIGATOR(S)

8. Miss F. K. Putney
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To elucidate one of the specific chemical mechanisms by which important biological compounds undergo radiochemical decomposition in relation to products formed. In this case: the formation of β-keto acids from β-hydroxy acids.

Methods Employed: The usual analytical chemical methods.

Major Findings: It has been found that dissolved molecular oxygen has a specific directing influence on the radiochemical decomposition of some β-hydroxy aliphatic organic acids in aqueous solution. We have been studying malic acid, β-OH butyric acid and citric acid.

With malate and β-OH butyrate, systems our data demonstrate that the initial radiochemical reaction is dehydrogenation of the solute molecule by the OH radical. Because a high yield of the corresponding β-keto acid is obtained it must be concluded that a chemical reaction of molecular oxygen and the solute radical for form organo-peroxide intermediates occurs.

Formation of this organo-peroxide intermediate allows the loss of a second hydrogen atom thus allowing the carbon to carbon structure to remain intact.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Studies with citrate indicate a similar mechanism. However, a more stable organo-peroxide intermediate is formed which very slowly breaks down to give an as yet unknown compound. A considerable number of compounds have been ruled out in attempts to identify this end product. Identification studies will continue until some explanation of the mechanism can be given. No attempt is being made to obtain a quantitative radiochemical balance between compounds formed and solute destroyed.

Studies in non-oxygenated solutions conclusively reveal that totally different mechanisms are involved in the radiochemical decomposition of the solute under study.

Significance to Cancer Research: Knowledge regarding the specific chemical mechanisms by which ionizing radiation may decompose biological compounds in aqueous solution should contribute greatly to the understanding of the physiological effects of ionizing radiations.

These studies are of importance too in that they provide another means of studying the behavior of organo-peroxide compounds which appear to be of considerable importance in biochemical function.

Proposed course of project: The purpose of the studies is to determine the general mechanism involved in the oxygen directed radiochemical reactions. When identification of the citrate compound is established it is doubtful that this work will be continued.

10. MC-620
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	[illegible]	1	1	2	1/3	1	1-1/3	
FY 1956	0	--	--	--	--	--	--	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE) 2. Radiation Branch (LABORATORY OR BRANCH)

3. Radiation Physiology Section (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. ZC1-621 (SERIAL NO.)

6. Studies on the Low Temperature Recovery of X-irradiated E. coli Cells.
PROJECT TITLE

7. A. W. Pratt
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Studies on the low temperature recovery of x-irradiated E. coli cells. The actual experimental results were obtained during a previous reporting period. During this reporting period we have completed a mathematical analysis of the results which within limits defines the magnitude of the various processes which result in the experimentally observed survival ratios.

Methods Employed: Routine methods used in the study.

Major Findings: It was concluded that:

(1) A depressed metabolic rate following x-irradiation exposure will serve to arrest the lethal effects of x-irradiation in a large number of cells, and with time of storage in the cold a significant number of cells can recover the vital function of cell division.

(2) The recovery mechanism is intrinsic in the cell -- the best recovery response is obtained in the presence of a complete media.

Significance to Cancer Research: The significance of these findings relates to the overall general radiobiology program of the Institute in attempting to define the response of biological specimens to ionizing radiation. The analysis is important in that it demonstrates the practicability of constructing a working hypothesis which can experimentally be rigorously tested thus affording another method for definition of mechanism.

Proposed course of project: No further work is contemplated.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

10. NC(-621
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$9105	1	--	1	1/3	--	1/3	
FY 1956	0	--	--	--	--	--	--	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

15. NCI-621
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

A. W. Pratt, W. S. Moos and M. Eden: Study of recovery at low temperature of x-irradiated E. coli Cells. J. Nat. Cancer Inst. In press. Probable date of appearance, February, 1955.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Radiation Branch
LABORATORY OR BRANCH

3. Radiation Physiology Section
SECTION OR SERVICE

4.
LOCATION (IF OTHER THAN BETHESDA)

5. ZC-[illegible]
SERIAL NO.

6. Mass Spectrometer Program
PROJECT TITLE

7. W. C. White
PRINCIPAL INVESTIGATOR(S)

8. A. W. Pratt
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

(1) The instrument is in continuous routine use for calibration and monitoring of the energy metabolism instruments.

(2) The research program for the mass spectrometer is devoted to the study of the deviations in the effusion rate of gases from that predicted by Graham's Law. (Graham's Law states that the effusion rate of a gas is proportional to the square root of molecular weight).

We hope that the determination of unknown gases obtained from biological systems in minute quantities may be accurately accomplished by mass spectrometric techniques.

Methods Employed: Mass spectrometric techniques.

Major Findings: It was found in the determination of the molecular weight of gases by measuring their effusion rates in the inlet system of the mass spectrometer that the rates of diffusion of various gases were not constants over the range of pressures from 1 to 100 microns. Such behavior prevents the exact determination of the molecular weight of

unknown gases obtained from biological systems. The effusion rate was shown to agree with Knudson's empirical formula:

$$T = ap + b\frac{1 + C_1P}{1 + C_2P} \quad \text{where}$$

$$T = \frac{\ln P_1 - \ln P_2}{t_2 - t_1}$$

p = pressure
$a_1 b_1 c_1$ and c_2 are constants.

When the effusion rates are extrapolated to zero pressure the molecular weights and effusion rates agree with Graham's Law.

Significance to Cancer Research: By these basic physical studies we are attempting to utilize the considerable potential of the mass spectrometer in biological research. We may also be able to aid in the further definition of a fundamental physical law.

Proposed course of project: We will continue to accumulate effusiometric data on gas systems including those related to biological systems.

10. NC1-622
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$14,591	1	1	2	1	1	2	
FY 1956	$14,593	1	1	2	1	1	2	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. General Radiobiology Section — SECTION OR SERVICE
4. ____ — LOCATION (IF OTHER THAN BETHESDA)
5. ZRCI-623 — SERIAL NO

6. Effects of Radiation on Central Nervous System
PROJECT TITLE

7. Howard L. Andrews and Kirkland C. Brace
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To study the effects of radiation on the central nervous system.

Methods employed: Animals will be given relatively high doses of X-rays of various energies so that the depth dose delivered to low lying centers can be varied. Results of the irradiation will be studied by the electroencephalograph and by the determination of various sensory thresholds. The action of pharmacologic agents will also be studied.

Major findings: We have shown that pentobarbital, given before a radiation dose of 7,500 r, completely suppresses the convulsions regularly seen at this dosage, and lengthens the survival time from about 20 to 80 hours. With pentobarbital the 7,500 r animals appear identical with those untreated and receiving 5,000 r or less. Even at 15,000 r pentobarbital eliminates all signs of hyperexcitability and increases the survival time from 17 to 39 hours. Diphenyl hydantoinate is equally effective in suppressing postradiation convulsions, but has only a slight effect on survival time. Morphine, cysteine and lecithin are without effect on either the symptoms or the survival time.

Significance to Cancer research: Any information on the biological effects of radiation should lead to a better understanding of the mechanisms of radiation therapy.

Proposed course of project: By altering the depth dose-skin dose ratio, to attempt to localize areas in the brain or brain stem responsible for the observed symptoms. Pharmacological agents and studies on sensitivity thresholds will also be of help in determining areas of greater or less radiation sensitivity.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

10. Me1-623
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ [illegible]	1	0	1	2/3	2/3	1-1/3	
FY 1956	$12,[illegible]	1	0	1	1	2/3	1-2/3	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM (Cont'd)

15. NC-623
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Modification of the Early Radiation Death in Guinea Pigs. Howard L. Andrews, Kirkland C. Brace and Ervin J. Liljegren. Submitted to Proc. Soc. Exp. Biol. and Med.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. General Radiobiology Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-624 — SERIAL NO

6. Biological Effects of Radiation at High Dose-Rates — PROJECT TITLE

7. Howard L. Andrews and Kirkland C. Brace — PRINCIPAL INVESTIGATOR(S)

8. Raymond E. Murphy — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To establish similarities or differences between the biological effects of radiation given at normal rates (50-100 r/min.) and the effects when given at dose rates of 1,000 r/min or more.

Methods employed: Using the 3 Mev generator the entire dose range from 28 day lethal to perhaps 100 times lethal will be investigated. Survival times, mortality data, and other pertinent physiological variables will be studied.

Major findings: A new project.

Significance to Cancer research: The effects of high dose rates are unknown because all hitherto available sources have outputs falling in a rather limited range. There may be nothing worthwhile in high dose-rate therapy but its possibilities should be explored.

Proposed course of project: When methods of accurate dosimetry are developed mice and guinea pigs will be exposed to from 500 to 50,000 r of X-rays given at a variety of dose rates from 100 r/min. to the maximum possible. The animals will be studied by established methods and compared.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

10. MCI-624
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	C	0	0	0	0	0	0	
FY 1956	$ 12,[illegible]	2	1	3	1	2/3	1-2/3	

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]
REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. General Radiobiology Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. ZC-325 — SERIAL NO

Early Radiation Death in Guinea Pigs
PROJECT TITLE

K. C. Brace and H. L. Andrews
PRINCIPAL INVESTIGATOR(S)

E. C. Thompson
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objectives: To study the effect of massive doses of radiation and determine the cause of death.

Methods employed: Guinea pigs were exposed to whole body X-irradiation at doses between 1,000 and 25,000 r at a constant dose rate of 50 r per minute. The effects of the radiation were followed with electroencephalograms, electrocardiograms, complete blood counts, blood chemistry and survival time.

Major findings: There is a sharp transition in survival time at a dose of 6,000 r. At doses less than 6,000 r, the survival time exceeds 100 hours. Doses greater than 6,000 r cause death in less than 30 hours. Irradiation of the head only produced the same effects as whole body exposure. There is a severe granulocytosis during the first few hours after exposure but there are no significant changes in the blood chemistry.

Significance to Cancer research: The brain has long been considered to be insensitive to irradiation. This work shows that there is a direct effect of radiation on the central nervous system which may be the cause of death. This sensitivity of the central nervous system to radiation is important in the treatment of cancer in this area.

Proposed course of project: A portion of the next year will be devoted to determining what part of the brain constitutes the sensitive area. We expect to locally irradiate various areas to determine which is the most sensitive. The study of neuropathology of radiation has been started and will continue on during the next year.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

10. NC/-625
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	[illegible]	2	1	2	1	1/3	1-1/3	
FY 1956	[illegible]	2	1	3	1	1/2	1-1/2	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐
REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM (Cont'd)

15. NC/-625
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Early Radiation Death in Guinea Pigs. Am. J. Physiol., Nov. 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

None

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Radiation Branch
LABORATORY OR BRANCH

3. General Radiobiology Section
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. ZC-626
SERIAL NO

6. Life Span of the Turtle Erythrocyte
PROJECT TITLE

7. K. C. Brace and P. G. Altland
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: Measurement of the life span of the turtle erythrocyte.

Methods employed: The turtle erythrocytes were incubated in vitro with glycine-2-C^{14} to determine if the glycine is utilized in the poikilotherms similar to the homoiotherms. The uptake and incorporation of glycine into the erythrocytes was determined by measuring the radioactivity. Glycine was injected intraperitoneally into the turtles and the specific activity of the erythrocyte determined at various intervals thereafter.

Major findings: The life span of the turtle as determined by this method exceeds 11 months. This far exceeds the life span of any of the homoiotherms that have been measured.

Significance to Cancer research: This finding explains the peculiar resistance of the turtle erythrocyte to X-irradiation.

Proposed course of project: We plan to extend this study to a species of the amphibian to determine if this long life span is characteristic of all poikilotherms.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

10. NCI-626
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 19,525	2	0	2	1	0	1	
FY 1956	$ 19,525	2	0	2	1	0	1	

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

National Institute of Arthritis and Metabolic Diseases

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. General Radiobiology Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. Z1-G2 — SERIAL NO

6. Change in Tissue Constituents by Radiation
PROJECT TITLE

7. Howard L. Andrews and Ervin J. Liljegren
PRINCIPAL INVESTIGATOR(S)

8. none
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine the changes in amino acid concentrations produced in various body tissues by X-irradiation.

Methods employed: Using separation columns and paper chromatography assays of various tissues will be made for a series of amino acids. Radiated and normal animals will be compared.

Major findings: Preliminary experiments indicate substantial changes in these concentrations.

Significance to Cancer research: Any increase in knowledge of the biological effects of radiation is of interest in radiation therapy.

Proposed course of project: The next year should see considerable progress and results on a large number of amino acids and tissues are to be expected.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

10. MCI-627
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$4755		1	1	0	1/3	1/3	
FY 1956	$ 5315		1	1	0	1	1	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. General Radiobiology Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. ZC-628 — SERIAL NO

6. Dosimetry of high intensity 3 Mev X-rays.
PROJECT TITLE

7. Howard L. Andrews
PRINCIPAL INVESTIGATOR(S)

8. Raymond E. Murphy
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To develop methods for measuring the X-radiation produced by the 3 Mev Van de Graaff generator.

Methods employed: Exact methods must be determined. It is proposed to start by a comparison of chemical dosimeters, photographic emulsions and ionization chambers.

Major findings: This is a new project. The only work done so far has involved putting the generator into workable form.

Significance to Cancer research: This has only a secondary significance by making possible quantitative measurements of radiation which will be of possible interest in cancer therapy.

Proposed course of project: It is hoped that ionization chambers can be developed to measure the X-ray intensities anticipated from this generator. Chemical dosimeters appear to be dose-rate independent, but are not easy to handle for routine dosimetry. They can be used as a standard with which other instruments can be compared. If satisfactory methods can be devised depth dose studies will be carried out with the 3 Mev beam and the dosimetry of high intensity pulses will be investigated.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

10. Ncf-625
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 4,8[illegible]	1	1	2	1/3	1/3	2/3	
FY 1956	$ 6,[illegible]37	1	1	2	1/3	2/3	1	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. Radiation Branch
LABORATORY OR BRANCH

3. General Radiobiology Section
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. NC/-629
SERIAL NO

6. The effect of ionizing radiation on amino acids.
PROJECT TITLE

7. Charles R. Maxwell
PRINCIPAL INVESTIGATOR(S)

8. Dorothy C. Peterson
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: The objective of this project is to determine the mechanism of the chemical reactions induced by ionizing radiation in aqueous solutions of amino acids. This is part of a long range program to accumulate information on simple systems of biological interest so that general principles may be ascertained and applied to complex systems which are not amenable to thorough, direct investigation.

Methods employed: Solutions of amino acids are irradiated with ionizing radiation and then analyzed for the products formed. Since the products are sensitive to radiation it is necessary to determine the yield of each product as a function of dose to as low a dose as possible and to extrapolate these values to obtain the initial yield of the product at very low doses before secondary reactions distort the picture.

Most of the study is done with 50 KV X-rays as the ionizing radiation. The effect of dose rate and ion density is investigated by irradiating with electrons and alpha particles. Protons and neutrons will probably be used in the future.

Major findings: The yield-dose data for H_2 and CO_2 has been completed this year. These data, plus the data obtained previously, have enabled us to make a material balance on the irradiated solutions and to propose four gross chemical reactions to account for the observed effects. These reactions seem to completely describe the effect on glycine but there seems to be an additional unknown reaction occurring with alanine.

The initial chemical effect produced by 160 KV electrons at a dose rate of 2,500,000 r/min. and 50 KV X-rays at a dose rate of 1,600 r/min. has been

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

shown to be the same. The role of secondary effects with large doses is, however, quantitatively different.

A large part of the year has been spent in developing and becoming proficient in the use of special micro analytical techniques necessary for the determination of the products formed. This phase seems to be almost complete.

Significance to Cancer research: Although not directly connected with Cancer research, it is of great interest because it seeks an understanding of the mechanism for the effects of radiation which is used empirically as a tool in the clinical treatment and laboratory study of cancer.

Proposed course of project: The work in Fiscal 1956 will be a continuation of the work of Fiscal 1955 and will be directed toward the elucidation of the mechanism of the reactions induced by radiation.

Specifically:

(a) The role of the H free radical will be investigated by a completion of the study now in progress on the effect of dissolved oxygen.

(b) The role of the OH free radical will be investigated by a completion of the study now in progress on the effect of dissolved hydrogen.

(c) The role of H_2O_2 (a known product of pure water) will be investigated by irradiations in the presence of dissolved catalase which should enzymatically destroy the H_2O_2.

(d) Measurements upon the influence of glycine concentration and of temperature upon the relative frequency of the various reactions will be refined.

(e) The study on the effect of alpha particles upon glycine will be resumed.

PROJECT REPORT FORM (Cont'd)

10. M21-629
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 23,216	2	1	3	2	1	3	
FY 1956	$ 23,360	2	1	3	2	1	3	

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

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14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

15. 701-627
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

The effect of ionizing radiation on amino acids. I. The effect of X-rays on aqueous solutions of glycine. Charles R. Maxwell, Dorothy C. Peterson and Norman E. Sharpless. Radiation Research, Dec. 1954.

The effect of ionizing radiation on amino acids. II. The effect of X-rays on aqueous solutions of alanine. Norman E. Sharpless, Alberta E. Blair and Charles R. Maxwell. Radiation Research (in press). Probably out in Feb. 1955.

The effect of ionizing radiation on amino acids. III. The effect of electron irradiation on aqueous solutions of glycine. Charles R. Maxwell, Dorothy C. Peterson and William C. White. Radiation Research (in press). Probably out in Mar. 1955.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. General Radiobiology Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. ZRC1-630 — SERIAL NO

6. Immune reactions in irradiated animals — PROJECT TITLE

7. Falconer Smith — PRINCIPAL INVESTIGATOR(S)

8. Marie M. Grenan, H. Jeanette Ruth, Hazel P. Gump — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objectives in this project are (1) to characterize quantitatively the recovery of the immune process following irradiation; (2) to determine the importance of bacterial immunity in irradiated animals and (3) to study the biological effects of irradiation on specific tissue antibodies.

Methods employed: Animals were immunized before or after irradiation with standard amounts of antigen. The rates of antibody production and peak titers were quantitatively estimated in mice, rats and rabbits. Similar tests have been carried out with mice and rabbits treated therapeutically with bone-marrow and spleen homogenate following irradiation.

Mice and rats have been immunized with commonly occurring enteric bacteria and the effect of this immunity on survival after LD_{50} to LD_{90} doses of radiation has been studied. In addition, the effect of bacterial immunity on survival after LD_5 doses of radiation in mice and rats given experimental infections was observed.

Guinea pigs have been immunized with suspensions of rabbit platelets and rabbits have been immunized with mouse leucocytes. The resultant antiserums are used in studying the reactions of the homologous species after irradiation.

Major findings: The major findings have been (1) to demonstrate that survival of rats and rabbits following LD_{50} to LD_{90} doses of radiation is independent of their capacity to produce antibody (F. Smith and Gump); (2) production of antibody in mice has not recovered by 30 days after irradiation (LD_5)

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

even
/ with bone-marrow or spleen homogenate therapy (F Smith and Ruth); (3) that preirradiation immunity to certain enteric bacteria results in a marked protection to mice and rats experimentally infected after LD_5 doses of radiation but does not improve survival in mice (not experimentally infected) after LD_{50} to LD_{90} doses of radiation (F. Smith, W. W. Smith, Gonshery and Grenan; (4) 1/ antiserum prepared against mouse leucocytes is lethal to normal mice when injected intravenously. Irradiation (LD_{40}) markedly increases the sensitivity of mice to this antiserum. Heat (1 hr. at 50° C) destroys the lethality of the antileucocyte serum and "absorbing out" with mouse leucocytes diminishes its potency (F. Smith, Gump and Grenan).

1/ Item (4) is considered confidential.

Significance to Cancer research: Immunochemical procedures help to clarify the nature of radiation injury at the cellular level and may also yield some information on the problem of tissues or cells responsible for the elaboration of antibodies.

Proposed course of the project: It is proposed to continue the study of hemolysin production in irradiated mice and rabbits using lysed sheep cells as antigen (F. Smith and Ruth) and to obtain additional data on the tissue antiserums in both irradiated and normal animals, quantifying, where possible, the antigens involved (F. Smith, Gump and Grenan).

10. 71C1-630
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$16,748	1	2	3	2/3 ~~0.6~~	2	2 2/3	
FY 1956	$25,229	1	3	4	1	3	4	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No Entries Under 15, 16 & 17

PROJECT REPORT FORM (Cont'd)

15. 2-C-1-630
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Specific Antibody Titer and Survival after Total-Body X-Irradiation. Proc. Soc. Exp. Biol. and Med., 85:412, 1954. Falconer Smith and Hazel P. Gump.

Effect of Immunity on Resistance to Infection in Irradiated Mice and Rats. Proc. Soc. Exp. Biol. and Med., 87:23, 1954. Falconer Smith, Willie W. Smith, L. Gonshery and Marie M. Grenan.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. General Radiobiology Section — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. ZC1-63 — SERIAL NO.

6. Effect of parenterally injected inorganic particulate irritants on survival of irradiated animals.
PROJECT TITLE

7. Falconer Smith and Willie W. Smith
PRINCIPAL INVESTIGATOR(S)

8. Marie M. Grenan, H. Jeanette Ruth, Ilo M. Alderman
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: To study the effect of inflammation produced by particulate irritants on survival and resistance to infection in irradiated animals.

Methods employed: Survival rates were observed in animals given suspensions containing particulate matter either before or after exposure to midlethal irradiation. Limestone, calcite, calcium carbonate, quartz and glass particles of varying size and amount were tested as to their effect on survival. Lethally irradiated, particulate treated mice were studied for evidences of altered differential leucocyte counts in the blood or peritoneal exudate and for infecting organisms, while sublethally irradiated mice were experimentally infected with Pseudomonas and Proteus.

Major findings: Intraperitoneal injections of inorganic particulate matter increased the survival of mice exposed to LD_{50} to LD_{90} X-irradiation by about 24 mice per 100. Essentially the same degree of protection was observed in guinea pigs and hamsters but was not apparent in rats.

Sublethally irradiated mice treated with particulate matter had a higher resistance to bacterial infection than mice not so treated and preirradiation immunization with homologous bacteria enhanced the protective effect of the particulate.

Significance to Cancer research: The results of these studies help to elucidate the process of recovery from severe radiation damage.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Proposed course of the project: This project is approaching completion. Two additional experiments are planned, one on the route of administration of the experimental infections and one comparing the rates of disappearance of intravenously injected bacteria in particulate treated mice with their irradiated controls.

10. Nc(-631
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$8,204	2	2	4	0.6 1/3	1	1 1/3 1.4	
FY 1956	"	0	0	0	0	0	0	

12. BUDGET ACTIVITY:

RESEARCH ☑ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

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14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. General Radiobiology — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. [illegible] — SERIAL NO

6. Studies of defense mechanisms in animals damaged by and recovering from X-irradiation.
PROJECT TITLE

7. Willie W. Smith
PRINCIPAL INVESTIGATOR(S)

8. R. Q. Marston (Army - A.F.S.W.P.), L. Gonshery (A.F.S.W.P.), Ilo Alderman, H. J. Ruth (A.F.S.W.P.), J. Cornfield (O.D.)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To study mechanisms of defense against death from infection in irradiated animals, means of increasing survival by improving resistance to infection, and the physiological events leading to recovery.

Methods: (1) Mice given sublethal irradiation, in some groups followed by an intravenous injection of spleen homogenate, were subsequently challenged with Pseudomonas or Proteus, and differential leucocyte counts made just prior to challenge. The period from 5 to 19 days following irradiation was studied in this way. (2) Litters of mice were exposed to midlethal radiation, differential leucocyte counts made 4 and 7 days later, and bacterial cultures of heart blood made on animals that failed to survive.

Major Findings: (1) Resistance to bacterial challenge was closely associated with granulocyte count and was not related to lymphocyte count either during normal recovery or during the advanced recovery induced by spleen homogenate injection. Either granulocytes, or specific constituents of the recovery process more closely linked with granulocyte than with lymphocyte recovery, must be responsible for this association. (2) Survival of midlethally irradiated mice was associated with granulocyte count (but not with lymphocyte count) taken either 4 or 7 days after irradiation. The clustering of survival in litters was highly significant (confirming the observation of Kaplan and Brown) in contrast to the clustering of relatively high granulocyte counts. The occurrence of bacterial species (with the exception of E. coli) in postmortem heart blood was significantly influenced by litter, which could account for at

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

least part of survival-litter association.

Significance to the program of the Institute: These studies not only help to elucidate the response of the animal to X-irradiation but are also concerned with functional aspects of regeneration of severely damaged hemapoietic tissues.

Proposed course of the project: This project has been completed.

PROJECT REPORT FORM (Cont'd)

10. C-4954
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955		1	3	4	0.5	0.5	1.0	
FY 1956		0	0	0	0	0	0	

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Funds and personnel (1 professional, 6 other) provided Jan. 1-June 30, 1954 by the Department of Defense. Estimated man years from this source for this project: Prof., 0.5; other, 1.5; total, 2.0.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

15. NC/-632
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Granulocyte count, resistance to experimental infection and spleen homogenate treatment in irradiated mice. Willie W. Smith, Robert Q. Marston, H. Jeanette Ruth and Jerome Cornfield. Am. J. Physiol. 178:288, 1954.

Effect of granulocyte count and litter on survival of irradiated mice. Willie W. Smith, Leon Gonshery, Ilo M. Alderman and Jerome Cornfield. Am. J. Physiol. 178:474, 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

Invitation to participate in conference on physiological effects of radiation, Argonne National Laboratory, Oct., 1954.

Invitation by the American Physiological Society to serve as chairman of a radiation section, Federation Meetings, April 1955.

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. General Radiobiology — SECTION OR SERVICE
4. ____ — LOCATION (IF OTHER THAN BETHESDA)
5. ZC-433 — SERIAL NO

6. Functional studies of the hyperplastic spleen of mice recovering from radiation damage.
PROJECT TITLE

7. Willie W. Smith
PRINCIPAL INVESTIGATOR(S)

8. Ilo M. Alderman
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To obtain a better understanding of functional aspects of recovery following radiation damage, particularly relating to the hematopoietic system.

Methods employed: (1) Resistance to Pseudomonas challenge and differential leucocyte counts were studied in intact and splenectomized mice 15 and 20 days after irradiation (600 r, streptomycin). (2) Leucocyte mobilization following Pseudomonas injection was studied 12, 14 and 16 days following sublethal irradiation (475 r). (3) Homogenates of spleens from immature and recovering irradiated mice were compared for ability to induce recovery in irradiated recipients (750 r).

Major findings: (1) During recovery from sublethal radiation intact mice were more resistant to experimental infection than were splenectomized mice. Granulocyte counts were the same in both groups 15 days after irradiation (spleen weight 60 mg.) but were about 3 times as high in the intact mice 20 days after irradiation (spleen weight 150 mg.). The difference in granulocyte count does not account for the difference in resistance to infection. (2) Granulocyte counts for the study on mobilization have not been completed. (3) Spleens from recovering irradiated adult mice were just as effective as spleens from immature nonirradiated mice as a source of protective homogenate. (Reports of others show this not to be the case with nonirradiated adult spleens). This is associated with the degree of hematopoietic activity seen in the several types of spleen.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Significance to the program of the Cancer Institute: These experiments are of interest in that they relate to the problem of hyperplasia following cellular damage.

Proposed course of project: Essentially complete except for final report.

10. Inc/-633
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$5,940	1	3	4	~~0.3~~ 1/3	~~0.5~~ 1/2	~~0.5~~ 5/6	
FY 1956		0	0	0	0	0	0	

12. BUDGET ACTIVITY:

RESEARCH ☑ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Funds and personnel (1 professional, 6 other) provided Jan. 1-June 30, 1954 by the Department of Defense. Estimated man years provided from this source for this project: Prof., 0.3; other, 0.5, total, 0.5.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. General Radiobiology — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. ZC-634 — SERIAL NO

6. Long-term effects of radiation in supralethally irradiated hamsters treated with streptomycin or bone marrow.
PROJECT TITLE

7. Willie W. Smith
PRINCIPAL INVESTIGATOR(S)

8. Pathological studies by Dr. Katharine C. Snell.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To study long-term effects of relatively high radiation exposures. This becomes possible and increasingly important as methods for preventing radiation death from agranulocytosis, hemorrhage and anemia are developed.

Methods employed: Hamsters exposed to radiation were treated with streptomycin (790 r) or injected with homogenate of marrow and spleen (1050, 1160 r). Hematological and bacteriological studies were made, and pathological examinations made by Dr. Snell as the animals became moribund or died.

Major findings: Although survival 28 days following irradiation was roughly the same in the three groups (40-60%), subsequent to that time deaths continued to occur, the rate being associated with the radiation exposure rather than with 28-day survival. A variety of pathological changes (including beginning cataract) were reported by Dr. Snell.

Significance to the program of the Cancer Institute: Radiation is known to give rise to neoplastic changes, and such changes are to be expected in this type of experiment. Of more immediate significance, however, is the indication that hematopoietic recovery (induced by bone marrow) will prove not to mitigate the radiation damage which becomes apparent one to twelve months after exposure.

Proposed course of project: These studies seem to us to be vitally important, and their continuation desirable. This is to some extent contingent upon the availability of facilities and continued collaboration of a pathologist.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

10. NC(-034
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$5772	1	3	4	1/3 ~~0.3~~	1/2 0.5	5/6 0.5	
FY 1956	$9456	1	3	4	1/2 ~~0.5~~	1.0	1 1/2 1.5	

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Funds and personnel (1 professional, 6 other) provided Jan. 1-June 30, 1954 by the Department of Defense. Estimated man years provided from this source for this project: Prof., 0.3; other, 0.5; total, 0.5.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NO ENTRIES FOR 15, 16 & 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. General Radiobiology — SECTION OR SERVICE
4. ____ — LOCATION (IF OTHER THAN BETHESDA)
5. 1C/-035 — SERIAL NO

6. Metabolic studies of the spleen during involution and hyperplasia following radiation exposure.
PROJECT TITLE

7. Willie W. Smith and Gilbert Ashwell (N.I.A.M.D.)
PRINCIPAL INVESTIGATOR(S)

8. -----
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To obtain a better understanding of the biochemical processes involved in destruction and regeneration of components of the hematopoietic system.

Methods employed: Spleen weights and ATPase content were determined in untreated and spleen homogenate-treated mice for 4 weeks following irradiation (640 r, streptomycin).

Major findings: Although spleen adenosine triphosphatase per mg. spleen increases during involution following irradiation, it decreases during hyperplasia. Spleen weights vary inversely with enzyme activity so that the total organ enzyme content varies only slightly while the spleen weight varies from 20 to 260 mg. Apparently the cells lost or gained are very poor in ATPase. The duration of involution and high ATPase activity was much shorter and subsequent hyperplasia and low ATPase activity were much less marked in homogenate-treated than in irradiated control mice.

Significance to the program of the Cancer Institute: These studies, when extended, may become of specific interest in connection with myeloid leukemia.

Proposed course of project: Spleens of normal immature and mature mice and of mice recovering from severe radiation damage (LD_{50}, streptomycin) will be analyzed for reactions involving ribulose, 3-keto pentose and xylulose, sugars recently discovered in the normal mouse spleen by Dr. Ashwell. We particularly

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

want to find out whether or not these newly-found reactions are associated with hematopoietic activity and the ability of spleen to induce hematopoietic recovery in irradiated recipients.

10. PHS-35
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	PHS-35	1	3	4	1/2 0.5	1/2 0.5	1 1.0	
FY 1956	-	0	0	0	0	0	0	

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Funds and personnel (1 professional, 6 other) provided Jan. 1-June 30, 1954 by the Department of Defense. Estimated man years provided from this source for this project: Prof., 0.3; other 0.5, total 0.5.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

15. [redacted]
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Spleen adenosine triphosphatase activity in irradiated mice treated with spleen homogenate. Willie W. Smith, William Anderson, Jr. and Gilbert Ashwell. Am. J. Physiol. 178:471, 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. Physical Chemistry Section — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. — SERIAL NO.

6. Physical Characterization of some Tumor Viruses and Cellular Formed Bodies — PROJECT TITLE

7. H. Kahler and B. J. Lloyd, Jr. — PRINCIPAL INVESTIGATOR(S)

8. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To increase the currently attained purification of some tumor inducing viruses and cell particulates and to determine some of their characteristics.

Methods Employed: The particulate is concentrated from a tissue extract by centrifugation procedures and studied by means of physico-chemical and electron microscopic technics.

Major Findings: The Rous Sarcoma virus was found to have a density of 1.15 in sucrose solutions. With the addition of D_2O to the solvent the density increased, indicating the replacement of H_2O in the virus structure by D_2O. The sedimentation constant of the virus indicated a larger particle than has previously been determined. The Shope Fibroma virus was identified both in extracts and tissue slices and resembles the myxoma virus in morphology.

Significance to Cancer Research: The study of these viruses is important for cancer research because of their high potency in inducing tumors and because of their "natural" occurrence in the animal.

Proposed course of project: Proposed work next year includes further work on attempts to differentiate between the properties of myxoma and fibroma virus, further purification of tumor inducing viruses and some further studies on desoxyribonucleic acid.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

10. AC/-634
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$13,737	2	--	2	1-1/2	--	1-1/2	
FY 1956	13,943	2	--	2	1-1/2	--	1-1/2	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

15. TC/-636
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

B. J. Lloyd, Jr. and H. Kahler: Electron microscopy of the rabbit fibroma virus. J. Nat. Cancer Inst. In press.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

PROJECT REPORT FORM

December 17, 1954

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-6506 — SERIAL NO

6. Clinical Radiotherapy. — PROJECT TITLE

7. John D. Reeves, M.D. (and Clinton C. Powell, M.D. - after July 1, 1954) — PRINCIPAL INVESTIGATOR(S)

8. None — OTHER INVESTIGATORS

9. **PROJECT DESCRIPTION**

The first 3 months of the calendar year 1954 were spent by Dr. John D. Reeves in completing the gathering of data and plans at the Holt Radium Institute, Manchester, and the Royal Cancer Hospital, London, for the purpose of applying these in the Radiation Wing of the Clinical Center.

The remaining 9 months have been spent in carrying on the radiotherapy begun by Dr. Lewis G. Allen. Since April 1, 1954, 46 patients have received radiotherapy. (Some patients have had more than one course of therapy.) The great majority of these were treated on the 250 KV machine. Two were treated by outside consultants because of the unavailability of the appropriate equipment here. One had a radon implant with radon furnished from the Baltimore Public Health Service Hospital and one had radium therapy with radium borrowed from Dr. Ralph Caulk at Garfield Hospital. The total number of X-ray treatments given has been 949. In addition, there have been 30 formal consultations and perhaps thrice this number of informal consultations.

Two 250 KV constant potential X-ray therapy machines have been installed on specially designed ceiling mounts. Two 2 Mev Van de Graff generators have been installed but not calibrated as yet. A rotating chair is being installed as this report is written.

(In addition, from April through July, Dr. Reeves has also assumed a full-time responsibility in diagnostic radiology due to a shortage of personnel in that department, and since that time has continued to take emergency calls as a voluntary aid to the diagnostic department.)

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

9. (CONTINUED)

Continuing into calendar year 1955:

1. Completion of the ordering and installation of intermediate and superficial X-ray therapy apparatus.

2. Completion of the ordering of appropriate radium as previously proposed. This will require the design and construction of a special laboratory.

3. Calibration and integration of the 2 Mev Van de Graffs into the clinical program. Patient handling facilities will be necessary in the sub-sub-basement before the 2 Mev area can be fully utilized.

4. Obtaining approval and designing special facilities for the handling of radioactive isotopes and patients in which isotope therapy is indicated.

5. Continuing plans for the psychological aspects of radiation therapy and the improvement in appearance of all areas where patients may be handled, such that their approach to radiation therapy shall be at its psychological optimum.

PROJECT REPORT FORM (Cont'd)

10. LC/-650(a)C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$23,916	2	1	3	2	1	3	0
FY 1956	$53,054	4	3	7	4	3	7	0

12. BUDGET ACTIVITY:

RESEARCH	☑	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Mr. Robert Swain, Physicist, Baltimore Public Health Service Hospital, has kindly given us preliminary calibrations on the two 250 KV therapy units and has, on one occasion, supplied us with radon seeds.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Parallels clinical radiotherapy performed in other Public Health Service radiotherapy departments. (Research cannot be fully started until calibrations and physical as well as biological base lines are established.)

No entries for 15, 16, and 17

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Radiation Branch — LABORATORY OR BRANCH
3. Office of the Chief — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. ZC1-CS04-1 — SERIAL NO.

6. Clinical Radiation Therapy Service
PROJECT TITLE

7. Clinton C. Powell and John D. Reeves
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

The following report covers the activity of Dr. Clinton C. Powell from the period of July 1, 1954 through December 31, 1954.

Approximately 90% of this investigator's time was utilized in the procurement of equipment, its installation, calibration, and preliminary operation, and in radiation therapy given to patients. In all instances, these patients were treated as a service for other clinical Services of the Clinical Center. The majority of these patients were participating in other research projects of the Cancer Institute. The time required to carry out the above duties and the lack of availability of necessary equipment did not permit the establishment of any independent research projects. Much remains to be done in the way of procurement, calibration, and standardization of equipment and of operating procedures before independent research projects can be undertaken in the field of clinical radiation therapy. The above activities were carried out in cooperation with the other principal investigator available, Dr. John D. Reeves.

Approximately 5% of my time during the above period was used in connection with my commitments as a member of the American Hospital Association Committee on the Use of Radioisotopes in Hospitals. This newly formed committee is in the process of establishing recommendations which properly fall within its sphere. It has seemed proper that, as the only Medical Officer from the Public Health Service on this committee, I take an active role in all deliberations and decisions of this committee.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

NCI-650(b)C

9. PROJECT DESCRIPTION (Continued)

Approximately 5% of my time has been utilized in connection with Public Health Service off-site monitoring obligations. The Public Health Service has recently assumed increased obligation in connection with the evaluation of actual or potential hazards resulting in the off-site area from Atomic Energy Commission testing of nuclear devices at its Nevada Proving Ground. The importance of the assigned task, the relatively small pool of trained personnel for this project available to the Service, and the projected schedule of future activities in this field make it appear likely that a considerably greater portion of my time must be devoted to this activity during the remainder of the current fiscal year.

PROJECT REPORT FORM (Cont'd)

10. ZC1-65(4)C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$9,325	1	0	1	1	0	1	
FY 1956	$9,275	1	0	1	1	0	1	

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None, except that all necessary funds to cover expenses incurred directly in the carrying out of the Public Health Service off-site monitoring activities are provided by the U. S. Atomic Energy Commission.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

PROJECT REPORT FORM (Cont'd)

15. AC1-656 G.IC
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

None

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

Certification in Radiology by the American Board of Radiology, Inc., September 1954 (Dr. Clinton C. Powell).

PROJECT REPORT FORM

1. National Cancer Institute
INSTITUTE

2. General Medicine Branch
LABORATORY OR BRANCH

3. _____
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. ACL-7006(C)
SERIAL NO

6. Evaluation of the Riboflavin Analogue, U-2112
PROJECT TITLE

7. John L. Fahey
PRINCIPAL INVESTIGATOR(S)

8. Montague Lane
OTHER INVESTIGATORS

9. **PROJECT DESCRIPTION**

Objective: The objective of this project is to determine the pharmacology of 6, 7 - dimethyl - 9 - (2' - acetoxyethyl) isoalloxazine (U-2112) in human patients, expecially as related to neoplastic chemotherapeutic activity.

Methods Employed: Photofluorometric and chromatographic means of identifying and measuring flavins can be used to evaluate the chemical products of this compound in human tissues and products. Clinical observation will be correlated with laboratory findings.

Patient Material: The patient material utilized in the past year consisted of 5 patients who underwent clinical anti-tumor chemotherapeutic trial - an allied project carried out by Dr. Robert Sullivan.

Significance to Cancer Research: This compound has been demonstrated to have Riboflavin antagonist effects in bacterial systems and to interfere with the growth of certain mouse tumors. Riboflavin is essential for normal tissue activity.

The metabolic fate of this drug in the human patient will be important in evaluating clinical effects, as it is probable that the compound is changed in passage through the human patient.

Proposed course of Project: Initial activities will be directed at developing methods for quantitative recovery and identification of flavins and U-2112 from biological material. Clinical application of these methods to study the Pharmacology of the drug in patients can then be undertaken.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

10. RCI-700(a)C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$19,253	2	1	3	1½	1	2½	30
FY 1956	$19,231	2	1	3	1½	1	2½	60

12. BUDGET ACTIVITY:

RESEARCH ☆ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM

1. NCI
INSTITUTE

2. GENERAL MEDICINE BRANCH
LABORATORY OR BRANCH

3.
SECTION OR SERVICE

4.
LOCATION (IF OTHER THAN BETHESDA)

5. RCI-700(h)C
SERIAL NO

6. PRELIMINARY SCREENING OF CANCER CHEMOTHERAPEUTIC COMPOUNDS IN THE HUMAN BY LOCAL INJECTION METHOD
PROJECT TITLE

7. R. D. Sullivan
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Numerous testing methods have been developed for the pre-clinical screening of potential cancer chemotherapeutic agents. However, no single method or combination of methods has been altogether satisfactory in predicting which compounds or groups of compounds will have tumor-destroying activity in the human cancer patient. A method is developed that permits the preliminary screening of compounds in the human prior to more extensive clinical assessment.

Methods Employed: The technique embodies the intratumoral injection of test substances into cutaneous lesions of patients with primary and metastatic cancer. Patients with multiple, discrete, non-ulcerating intracutaneous lesions are selected for study. Minute doses of different test substances are injected into selected lesions without producing systemic effects. Other lesions are infiltrated with control vehicles or serve as uninjected controls. Serial biopsies are obtained at varying intervals to uncover qualitative morphologic changes. Correlations are made between local studies of a given agent and subsequent systemic administration of that agent.

Patient Material:

	No.	Average Stay - days
Adult males	1	*
Adult females	10	

*Studies on hospitalized patients are made during periods in which the hospitalization is required for conventional therapy or chemotherapeutic trials.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PRELIMINARY SCREENING OF CANCER CHEMOTHERAPEUTIC COMPOUNDS (cont'd)

		No.
Outpatient	No pts.	3
	No visits	25

Findings: Fifteen compounds were screened by this method in 12 patients. In patients with breast cancer results of local injection hormone studies correlated well with subsequent systemic hormone therapy. Several colchicine analogues on local injection (obtained from Dr. J. Leiter, Section of Chem. Pharm.) produced regression of lesions of one patient with mycosis fungoides and 2 patients with melanocarcinoma.

Significance to NCI: The development of more precise methods for screening compounds is paramount in a cancer chemotherapeutic program.

Proposed Course: Patients with accessible skin lesions will continue to be selected for local studies of potential anti-cancer compounds that are being considered for clinical trials.

PROJECT REPORT FORM (Cont'd)

10. ACI-700(6)C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$3,108	1	—	1	1/3	—	1/3	60
FY 1956	$4,638	1	—	1	1/2	—	1/2	120

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. NCI
INSTITUTE

2. GENERAL MEDICINE BRANCH,
LABORATORY OR BRANCH

3.
SECTION OR SERVICE

4.
LOCATION (IF OTHER THAN BETHESDA)

5. NCI-J006(C)
SERIAL NO

CLINICAL EVALUATION OF THE RIBOFLAVIN ANTAGONIST U-2112 AS A POTENTIAL ANTI-CANCER AGENT
PROJECT TITLE

R. D. SULLIVAN AND J. L. FAHEY
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objectives: To evaluate by human clinical trials the pharmacologic, toxicologic and possible tumor-destroying effects of the riboflavin analogue, U-2112.

Methods Employed: Patients with disseminated, incurable malignant neoplasms are selected for study after thorough evaluation of the clinical status of the patient as regards organ function and the extent of his neoplasm. Following a control period during which time a rotation diet, constant in riboflavin, is initiated and urine and fecal collections are made, patients are placed on increasing doses of the compound. Careful clinical observation and serial laboratory examinations are performed to uncover manifestations of toxicity and tumor inhibiting effects. Excretory patterns (i.e. blood levels, stool and urine output) are delineated as outlined in Project Report by Dr. John Fahey.

Patient Material: To date 8 patients have been studied as follows:

	No.	Average Stay Days
Adult Males	4	33
Adult Females	3	69
Children Males	1	10

Outpatients - None

Major Findings: Dosages as high as 3.0 gm/day of U-2112 have been administered for as long as 36 days without producing demonstrable evidence of riboflavin

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Clinical Evaluation of the Riboflavin Antagonist (cont'd)

deficiency, tumor-destroying effects, or drug toxicity. Five patients have died of their neoplasm and related complications, two patients have been discontinued from the study, and one patient is currently maintained on the drug.

Significance to NCI: Conventional methods of treating advanced cancer are totally without benefit in the vast majority of patients. The development of an effective chemotherapeutic agent offers the greatest hope in controlling the disease in its advanced stages.

Proposed Course of the Project: Hospitalized patients with disseminated epithelial and mesenchymal neoplasms will continue to be included in the clinical trials of this compound. Increasing dosages will be utilized until levels are attained that produce toxic manifestations without evidence of tumor-destroying effects.

PROJECT REPORT FORM (Cont'd)

10. NCI-700(C)C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$8,374	2	1	3	1/3	1	1 1/3	300
FY 1956	$8,410	2	1	3	1/3	1	1 1/3	1200

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. NCI
INSTITUTE

2. GENERAL MEDICINE BRANCH
LABORATORY OR BRANCH

3.
SECTION OR SERVICE

4.
LOCATION (IF OTHER THAN BETHESDA)

5. ZC1-700(6)C
SERIAL NO

6. A STUDY OF CANCER CHEMOTHERAPEUTIC AGENTS ADMINISTERED BY ARTERIAL CATHETER IN THE HUMAN CANCER PATIENT
PROJECT TITLE

7. R. D. SULLIVAN
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To administer various potential chemotherapeutic agents into the accessible arterial blood supply of malignant neoplasms in order to 1) determine if the arterial route has inherent advantages over other routes of administration with regard to anti-tumor effect, lessened systemic toxicity and other pharmacologic properties; and 2) to develop the arterial route as a method of screening cancer chemotherapeutic drugs.

Methods Employed: A simplified technique of arterial catheterization is employed which embodies the use of small diameter radio-opaque catheters introduced into accessible peripheral arteries through standard gauge needles. The catheters can then be manipulated to the desired site in the central or peripheral arterial tree. Single doses of drugs may be administered and the catheter withdrawn, or it may be affixed to a stopcock and left in situ for repeated therapy over periods of several weeks. Specimens of blood may be withdrawn for selected studies and angiograms of the organs catheterized may be obtained at varying intervals. Extensive clinical experience has indicated that complications of the technique are few and are of minor importance.

Patient Material:

		No. days
Adult Males	1	5
Adult Females	6	30

Outpatients - None

Major Findings: In two patients, pain resulting from tumor growth was relieved by arterial courses of nitrogen mustard. In 2 patients, courses of aza serine

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

A Study of Cancer Chemctherapeutic Agents (cont'd)

and 2-6 diamino-purine produced no demonstrable effect on their neoplasms. Oncolytic viruses were administered by this route in 2 patients (see project report by Drs. Waite and Smith). In one patient, a course of arterial demicolcin produced a remarkable skin lesion only within the distribution of the vessel into which the agent was delivered. Further studies of this compound are contemplated.

Significance to NCI: The study of potential cancer chemotherapeutic compounds offers one of the greatest therapeutic hopes of controlling advanced cancer. The development of newer screening methods is an integral part of such a program.

Proposed Course of Project: Hospitalized incurable cancer patients whose neoplasms have an accessible blood supply will be studied as outlined above. A variety of potential cancer chemotherapeutic agents will be screened.

PROJECT REPORT FORM (Cont'd)

10. NCI-700(d)C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$4,662	1	—	1	1/2	—	1/2	60
FY 1956	0	—	—	—	—	—	—	—

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. NCI (INSTITUTE) 2. GENERAL MEDICINE BRANCH (LABORATORY OR BRANCH)

3. (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-7016(C) (SERIAL NO)

6. METABOLISM OF NITROGEN, MINERALS AND VITAMINS IN PATIENTS WITH MALIGNANT DISEASE
PROJECT TITLE

7. Donald M. Watkin, M.D.
PRINCIPAL INVESTIGATOR(S)

8. Drs. Roy Hertz, Jesse Steinfeld, Donald Tschudy, John Fahey, Nicholas Leone, and Clinical Associates
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Investigation of the metabolic interrelationships of nitrogen, minerals and vitamins in the natural course of malignant disease and in the response of the disease to therapy.

Methods: Metabolic balance technique for the estimation of the net gain or loss of nitrogen, minerals, vitamins, etc. Electrophoretic studies of plasma proteins (Dr. Fahey). Radioisotope techniques for determination of red cell survival time and albumin turnover (Dr. Steinfeld). Isotopic nitrogen studies of the metabolism of nitrogenous metabolites (Dr. Tschudy).

Patient Material: Calendar 1954

January to May	3 patients
June through December	7 patients, 8 studies

Major Findings: Fluoride Exchange Studies - Fluoride exchange and its relation to nitrogen and mineral balance was examined in two leukemic patients with and one without chronic exposure to high fluoride concentrations in drinking water and in one normal subject. Preliminary data indicate more fluoride is excreted by patients with fluorosis.

Prostatic Studies - Balance studies in two patients with extensive metastatic prostatic malignancy treated by orchidectomy demonstrated a retention of calcium and protoplasmic constituents following orchidectomy.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

METABOLISM OF NITROGEN, ETC. (cont'd)

High protein feeding - Tube feeding of a very high protein diet was accompanied by rises in BUN in two patients.

Leukemia studies: Nitrogen, phosphorus, calcium and electrolyte balance we studied in three patients with chronic and one with acute leukemia. Preliminary data imply negative calcium balances. In the acute case, this was apparently altered by therapy.

Vitamin Studies - Vitamin excretion on a constant diet of known vitamin content were conducted in a relatively normal tube fed subject in an effort to demonstrate a relationship between tissue anabolism and vitamin retention. Tissue anabolism produced by androgen administration and by hyper alimentation after depletion has so far been accompanied by no significant change in vitamin excretion.

A similar study was attempted in a patient with cachexia due to cancer but was not completed because of the patient's rapid downhill course.

Red Cell Survival - In one patient with carcinoma of the prostate no prolongation of shortened red cell survival time occurred as a result of orchidectomy. In one patient with chronic leukemia red cell survival was markedly shortened.

Significance to Cancer Research:

Fluoride exchange studies - Investigates possible rate of fluoride in leukemia and ascertains its effect on the metabolism of other elements in leukemic and non-leukemic individuals.

Prostate Studies - Provide quantitative measures of the effectiveness of androgen withdrawal in altering the characteristics of widespread prostatic neoplasm.

High protein feeding: Points out the effects of high protein feedings on blood chemistries and demonstrates certain problems of tube feeding.

Leukemia studies: Shows the effect of chronic and acute leukemia on bone metabolism and shows how this may be altered by therapy.

Vitamin studies: No relationship to date has been shown between tissue anabolism and vitamin retention.

Red cell survival: Indicates diminished life span of erythrocytes in malignant disease.

METABOLISM OF NITROGEN, ETC. (cont'd)

Proposed Course of Project: Continuation of the investigation of metabolic interrelationships in malignancy and its response to therapy. Expansion of the program to involve further collaboration with chemotherapy, biochemistry and biophysics groups. Development and organization of a metabolic ward, metabolic kitchen and metabolism laboratory.

PROJECT REPORT FORM (Cont'd)

10. NCI-701(a) C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$35,655	1	5	6	1	5	6	600
FY 1956	$55,776	2	7	9	2	7	9	1500

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Endocrinology Branch, NCI
Dental Research, Dental Health Institute

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. NCI — INSTITUTE
2. General Medicine Branch — LABORATORY OR BRANCH
3. _____ — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-70(C) — SERIAL NO

6. A Study of Nitrogen Utilization in Human Tumor Patients by means of N^{15} labeled Amino Acids. — PROJECT TITLE

7. Dr. D. Tschudy; Dr. C. Baker; Dr. J. Steinfeld; Dr. D. Watkin; Mrs. A. Collins — PRINCIPAL INVESTIGATOR(S)

8. _____ — OTHER INVESTIGATORS

9. **PROJECT DESCRIPTION**

Objectives: To estimate the size of the metabolic pool (nitrogen) and rate of nitrogen incorporation into tissues of normals and cancer patients.

Methods: N^{15} aspartic acid is fed to the patient and four measurements are made: 1) total nitrogen excretion; 2) total urea excretion per day; 3) the size of the urea pool; 4) the N^{15} concentration in the urea pool at various times. By means of a number of equations it is possible to calculate from these data a value for the size of the nitrogen metabolic pool and the rate of protein synthesis per day, i.e. the amount of nitrogen incorporated into the total tissues per day.

Findings: None yet.

Significance to NCI: This type of study gives data (the calculations of which are based on assumptions which are not 100% correct) which can not be obtained except by isotope methods. The effects of cancer and dietary changes in cancer on the metabolic pool and rate of nitrogen incorporation into the overall organism can be estimated.

Future course: The above studies are preliminary and it is planned to extend the study at a later date to measurements of incorporation of N^{15} into specific tissues, including tumors. The geographic distribution of isotope in the tumor will be studied in addition to the determination of isotope content of a number of compounds in the tumor (following oral administration of N^{15} amino acid). Normal tissues (obtained at surgery) will also be studied along with serum proteins.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

10. AC/-701(L)C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$ 11,483	4	1	5	2/3	1	1 2/3	90
FY 1956	$ 14,573	4	1	5	1	1	2	180

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. NCI (INSTITUTE) 2. GENERAL MEDICINE BRANCH (LABORATORY OR BRANCH)

3. _____ (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5 NCI-70266 (SERIAL NO)

6. MEASUREMENT OF ALBUMIN "TURNOVER" AND TOTAL EXCHANGEABLE IN PATIENTS WITH CANCER
PROJECT TITLE

7. DR. J. L. STEINFELD
PRINCIPAL INVESTIGATOR(S)

8. _____
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To compare total exchangeable albumin with plasma albumin concentrations on patients with cancer. Other studies have shown that the circulating albumin represents approximately 40% of the albumin which is in equilibrium or exchange with I^{131} albumin injected intravenously in normals. Whether this percentage is altered in patients with neoplasms is as yet unknown and may be of importance in estimating the nutritional status of patients with advanced cancers.

Methods Employed: Injection of known amount (75-125 microcuries) of I^{131} labelled human serum albumin and measuring plasma and whole blood radioactivity at varying times following injection. Also radioactivity excreted into urine each 24 hours is measured. These values permit calculation of total exchangeable albumin and albumin "turnover" times.

Patient Material:		Days Each
Adult Males	10	30
Adult Females	10	30

Major Findings: Only 5 studies have been completed and no conclusions can as yet be drawn.

Significance to NCI: Will help in understanding the hypoalbuminemia (as determined by concentration of albumin in blood or plasma) in all stages of cancer.

Proposed Course: To perform at least fifteen such studies in an attempt to find some pattern.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

10. NCI-70260
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$7,295	1	1	2	½	1/2	1	600
FY 1956	$7,297	1	1	2	½	½	1	1000

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

NONE

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NONE

No entries for 15, 16, and 17

PROJECT REPORT FORM

1. NCI — INSTITUTE
2. GENERAL MEDICINE BRANCH — LABORATORY OR BRANCH
3. _____ — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-702[illegible] — SERIAL NO

6. METABOLIC STUDY OF SERUM PROTEINS: FATE OF INTRAVENOUSLY ADMINISTERED ALBUMIN — PROJECT TITLE

7. JOHN L. FAHEY, M.D. — PRINCIPAL INVESTIGATOR(S)

8. DRS. DONALD M. WATKIN AND J. L. STEINFELD — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: The objective of this project is to characterize the disappearance rate and distribution of the products of albumin loads administered intravenously to patients in various neoplastic and nutritional states.

Methods Employed: Complete metabolic balance techniques are to be utilized to follow Nitrogen and, if feasible, Calcium and Phosphorus Balances on selected patients. Intravascular distribution of albumin and other serum proteins will be measured by electrophoresis and radioactive iodinated albumin.

Patient Material: (1954)

	Number	Hospital Staff
Admissions: Adult Male	1	100 days

Major Findings: Preliminary evaluation of a just completed study on a patient with gastro-intestinal neoplasm in moderate nutritional deficiency reveals that about half of the albumin nitrogen is utilized for tissue and tumor needs. There is no evidence of a markedly increased rate of albumin degradation in this patient.

Significance to Cancer Research: Patients with neoplasm develop low serum albumin levels which may be directly due to the presence of cancer or an

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Intravenous albumin (cont'd)

indirect effect, attributable to malnutrition. Determination of the fate of intravenously administered albumin load under several conditions will help to clarify the problem. Nutritional evaluation of intravenously administered albumin can be undertaken at the same time.

Proposed Course of the Project: Repeated studies in two patients with carcinomas and in two patients with lymphomata will require at least a full year. Ideally, study should be carried out with the patient in good nutrition with the disease quiescent, later with discernable disease activity, again with the patient being moderately malnourished and finally in a markedly deficient nutritional state. Compromise, of course, will be made as necessary.

PROJECT REPORT FORM (Cont'd)

10. NC1-702 6C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$23,916	3	2	5	2	1	3	300
FY 1956	$23,868	3	2	5	2	1	3	900

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. NCI
INSTITUTE

2. GENERAL MEDICINE BRANCH
LABORATORY OR BRANCH

3. _____
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. 1C1-702(C)C
SERIAL NO

6. METABOLIC STUDY OF SERUM PROTEINS: DETERMINATION OF PROTEIN TURNOVER RATES BY MEANS OF ISOTOPE LABELED AMINO ACID INCORPORATION.
PROJECT TITLE

7. DR. JOHN L. FAHEY
PRINCIPAL INVESTIGATOR(S)

8. _____
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: The objective of this project is to characterize the synthesis and degradation of the serum proteins in patients with neoplastic disease.

Methods Employed: Plasma protein separation will be undertaken by cold ethanol fractionation (Method 10 of Cohn) and by cellulose column chromatography, when available. Labeling of proteins can be done by administration of N^{15} or S^{35} labeled amino acids. Determination of isotope content can be made by the usual means, and rates of protein degradation calculated.

{Patient Material / Major Findings} This is a new project.

Significance to Cancer Research: Patients with cancer may have abnormalities of the serum proteins. No studies have been reported utilizing in vivo labeling methods for characterizing serum protein metabolism. Correlation with clinical information and with therapy effects should provide information as to some of the factors controlling individual serum protein component levels in man.

Proposed Course of the Project: The initial efforts this year will be directed toward establishment of the cold ethanol fractionation procedure and introduction of paper electrophoresis. Isotope measurements will have to be arranged by collaboration or by development.

Subjects for study will be selected on the basis of diagnosis, clinical disease status, nutritional state and availability for serial studies.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

10. M(C)-7033(c) C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 7,395	2	1	3	1/2	1/2	1	0
FY 1956	$ 11,934	2	1	3	1	1/2	1 1/2	300

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. NCI
INSTITUTE

2. GENERAL MEDICINE BRANCH
LABORATORY OR BRANCH

3. _____
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. NCI-702(4)C
SERIAL NO

CHROMATOGRAPHY OF PROTEINS: SEPARATION AND ANALYSIS OF THE COMPONENTS OF BLOOD SERUM
PROJECT TITLE

Dr. J. L. Fahey
PRINCIPAL INVESTIGATOR(S)

H. Sober and E. Peterson (Laboratory of Biochemistry, NCI)
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objective: The objective of this project is to further characterize the serum proteins from normal and cancerous patients by application of new methods of protein separation.

Methods Employed: Drs. Sober and Peterson of the Laboratory of Biochemistry, NCI, have designed systems of substituted cellulose columns on which protein components move differentially under pH and salt gradients, in controlled temperature conditions (cold room). Fourteen serum protein components have been identified and characterized by electrophoretic and spectrophotometric means.

Patient Material: (1954) One patient, admitted primarily for another project, was utilized for this study.

Major Findings: During the past year a leukemic patient was studied at 6 different stages of disease activity. Component alterations were observed which indicate a correlation with disease state.

Significance to Cancer Research: The alteration of body protein metabolism in neoplastic states is reflected by serum protein changes. These have as yet been only grossly defined by other analytical means. Further identification and characterization of the serum proteins themselves are necessary in order that the causes of protein abnormality may be studied.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

10. NCI-702(d)C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 14,591	3	1	4	1	1	2	30
FY 1956	$ 14593	3	1	4	1	1	2	60

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

LABORATORY OF BIOCHEMISTRY, NCI

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. NCI
INSTITUTE

2. GENERAL MEDICINE BRANCH
LABORATORY OR BRANCH

3. _____
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. ZC-7026(C)
SERIAL NO

6. ELECTROPHORETIC STUDY OF THE SERUM PROTEINS IN NEOPLASTIC DISEASE STATES
PROJECT TITLE

7. JOHN L. FAHEY
PRINCIPAL INVESTIGATOR(S)

8. DR. J. L. STEINFELD
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: The objective of this project is to electrophoretically quantitate the serum protein changes occurring in neoplastic states and to correlate these changes with specific aspects of the clinical status of the patient.

Methods Employed: Moving boundary (Tiselius) electrophoretic methods describe the relative concentration of serum proteins. By the radioactive-iodinated albumin method (Dr. Steinfeld) plasma volume can be determined. Combining this information allows measurement of the intravascular quantity of individual protein components. Also, clinical evaluation of the type of disease, disease activity, metabolic and nutritional state of the host, and therapy employed is to be correlated with the serum protein status.

Patient Material: (1954) Patients admitted to the Clinical Center for other projects were utilized for this study.

Major Findings: About one hundred electrophoretic determinations have been completed in the past year. Correlation of clinical and electrophoretic data is not yet complete.

Significance to Cancer Research: A better delineation of the sequence of plasma protein changes in neoplastic disease is needed, for this is an aspect of tumor-host relationship which lends itself to quantitation. Previously reported studies have not been primarily designed so as to carry out sequential studies in the same patients. Also, the effects upon serum proteins of the anti-tumor therapeutic regimens is generally unknown.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Electrophoretic Study (cont'd)

Proposed Course of Project: Emphasis will continue to be upon serial determinations throughout the course of illness in the patients studied. Following evaluation of preliminary data areas of special interest will be selected for more intensive study. Included in this will be an evaluation of the changes induced by Cortisone and the newer steroid hormones.

Paper electrophoretic methods will be explored for the purpose of facilitating analytical procedures.

PROJECT REPORT FORM (Cont'd)

10. NC1-702(e)C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$14,591	2	2	4	1	1	2	350
FY 1956	$14,593	2	2	4	1	1	2	100

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

NONE

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: NONE

No entries for 14, 15, 16 and 17

PROJECT REPORT FORM

1. NCI
INSTITUTE

2. GENERAL MEDICINE BRANCH
LABORATORY OR BRANCH

3. _____
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. NCI-7036(C)
SERIAL NO

6. MEASUREMENT OF BLOOD VOLUME, RED CELL MASS AND TOTAL CIRCULATING ALBUMIN IN PATIENTS WITH CANCER
PROJECT TITLE

7. DR. J. L. STEINFELD
PRINCIPAL INVESTIGATOR(S)

8. _____
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Objectives: To determine whether there is a hypervolemia, normovolemia or hypovolemia in patients with various types of cancer, correlating blood volume with total body water as measured with antipyrine, in those instances where that is possible. Also, to determine the presence or absence of changes in total red cell mass rather than in concentration of hemoglobin and further to measure total albumin rather than concentrations of albumin in various types and stages of cancer.

B. Methods Employed: Injection of known small quantities (5-10 microcuries) of I^{131} labelled human serum albumin and measurement of its dilution as determined by counting samples of whole blood and plasma removed at twenty minutes. Together with hematocrit and albumin concentration this permits calculations of total circulating albumin, circulating red cell mass and plasma volume.

C. Patient Material: This study is performed on patients admitted for other study programs and so far has included adult males, adult females and children.

Major Findings:

Anemia as measured by total red cell mass and hypoalbuminemia as measured by total circulating albumin have not as yet been correlated with extent or type of cancer since too few values are available for any conclusions to be drawn.

(Cont'd on next page)

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PAGE 1 (cont'd)

Significance to NCI:

Studies such as this enable one to define anemia and hypoalbuminemia more clearly than concentration studies above. Further knowledge of blood volume and red cell mass is important for clinical management of many surgical and medical patients.

Proposed Course: To continue to measure blood volumes, red cell mass and circulating albumin in patients with cancer who have been admitted for other studies.

PROJECT REPORT FORM (Cont'd)

10. AC-703(a)C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$7295	1	1	2	1/2	1/2	1	90
FY 1956	$7297	1	1	2	1/2	1/2	1	60

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16 and 17

PROJECT REPORT FORM

1. NCI — INSTITUTE
2. GENERAL MEDICINE BRANCH — LABORATORY OR BRANCH
3. — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5 ZCI-703(C) — SERIAL NO

6. ESTIMATION OF RED CELL SURVIVAL IN PATIENTS WITH ADVANCED NEOPLASTIC DISEASE BEFORE AND AFTER TREATMENT — PROJECT TITLE

7. DR. J. L. STEINFELD — PRINCIPAL INVESTIGATOR(S)

8. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Objectives: To estimate the life of the red cell in patients with advanced cancer, first to determine if life span is short and second to ascertain what effect, if any, treatment of the neoplasm has on the life span of the red cell.

B. Methods: In vitro incubations of $Na_2Cr^{51}O_4$ with 50 cc of heparinized blood followed by removal of plasma, three sterile saline washes, reconstitution to 50 cc and auto-transfusion. Various blood samples are removed without stasis at intervals, the radioactivity per cm^3 of the blood measured and following appropriate corrections, the average life of the red cells are estimated.

C. Patient Material:

		No.	Average Stay in Days
Admissions:	Adult Males	8	20
	Adult Females	7	20
Outpatients-	November		(These studies may be pursued while other
	Number of Visits		studies are in progress.)

D. Major Findings: The average life of the red cells in all those patients with active neoplastic disease studied to date has been from 20 to 60 days, considerably shorter than the 100 to 120 days usually quoted as average red cell life. Thus far only one patient has been studied following therapy (orchidectomy for disseminated prostatic carcinoma) and no increase in red cell life was found.

(Cont'd on next page)

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PAGE 1 (cont'd)

E. Significance to NCI: This method may be useful as a parameter for estimating the effect of either conventional and/or new forms of cancer therapy if correlation is obtained between clinical improvement and increase in red cell life.

Proposed Course of Project: At least fifteen patients who have malignancies amenable to therapy and shortened average red cell life before therapy should be studied in pre- and post therapy states. Patients will be studied as in or out-patients depending on their clinical status and concomitant research on their disease.

PROJECT REPORT FORM (Cont'd)

10. NCI-703(h)C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$7,295	1	1	2	½	½	1	200
FY 1956	$7,297	1	1	2	½	½	1	600

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

The PHS Outpatient Clinic has cooperated by obtaining samples of blood for counting of radioactivity from patient whose employment was in the District of Columbia.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16 and 17

PROJECT REPORT FORM

1. NCI (INSTITUTE) 2. GENERAL MEDICINE BRANCH (LABORATORY OR BRANCH)

3. (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. ACI-704 C (SERIAL NO)

6. THE INFLUENCE OF DELTA-AMINOLEVULINIC ACID AND SOME OF ITS ANALOGUES ON TUMOR GROWTH (PROJECT TITLE)

7. DONALD TSCHUDY (PRINCIPAL INVESTIGATOR(S))

8. MILTON WINITZ (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives: Given in (6).

Methods: Compounds will be injected into human subcutaneous tumors and growth rates studied.

Findings: None as yet.

Significance to NCI: Delta-aminolevulinic acid has been shown to be an intermediate in the biosynthesis of pyrroles and can contribute its delta carbon atom as the ureido carbon of purines. This compound and some of its analogues are being synthesized in an attempt to determine whether human tumor growth can be stimulated by the compound and inhibited by its analogues. Since this compound is a substrate in certain "one carbon transfers," analogues of this compound might possibly augment the growth inhibitory effect of compounds already known to interfere with "one carbon transfer." It will be injected into human subcutaneous metastases in different concentrations alone and in combination with other agents. The effects on bacterial growth will also be studied.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

10. MC1-704 C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$4,863	2	1	3	1/3	1/3	2/3	60
FY 1956	$4,865	2	1	3	1/3	1/3	2/3	300

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16 and 17

PROJECT REPORT FORM

1. NCI — INSTITUTE
2. General Medicine Branch — LABORATORY OR BRANCH
3. _____ — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-705 — SERIAL NO

6. Aldolase and other Serum Enzymes in Patients with Cancer. — PROJECT TITLE

7. Dr. Laurens P. White — PRINCIPAL INVESTIGATOR(S)

8. _____ — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine factors responsible for elevation of aldolase in serum of some cancer patients; to correlate this elevation, if possible, with nitrogen balance, creatine and creatinine excretion, and observe effects of varied diet. Similar objectives pertain to measurement of other serum enzymes.

Methods: Biochemical measurement of blood and urine constituents
Metabolic balance studies

Patients: None yet. Will study patients with malignancy, muscular dystrophy and thyrotoxicosis as well as normals.

No results to date.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

10. NC1-705 C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$9325	1	—	1	1	0	1	0
FY 1956	$11,934	1	1	2	1	½	1½	60

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16 and 17

PROJECT REPORT FORM

1. NCI (INSTITUTE) 2. GENERAL MEDICINE BRANCH (LABORATORY OR BRANCH)

3. _____ (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. ZC-706 C (SERIAL NO)

6. STUDY OF THE VALUE OF A SKIN TEST USING POLYSACCHARIDE IN CANCER AND NON-MALIGNANT DISEASES (PROJECT TITLE)

7. DRS. MURRAY SHEAR AND JOHN H. TUOHY (PRINCIPAL INVESTIGATOR(S))

8. DR. R. LANE WROTH (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Using a solution containing a polysaccharide material from a bacterial source efforts are made to determine the reaction in the skin of patients with both malignant and non-malignant diseases to varying doses injected intracutaneously. By this means information as to the specificity of such reaction in cancer patients is being sought.

These efforts are in a sense an attempt to obtain confirmatory evidence of the previously reported successful application of a similar test using material from mammalian sources by at least one Japanese investigator.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

10. ACI-706 C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$4,662	3	—	3	½	—	½	50
FY 1956	$4,638	3	—	3	½	—	½	150

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

LABORATORY OF CHEMICAL PHARMACOLOGY, NCI

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: NONE

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. NCI
INSTITUTE

2. General Medicine Branch
LABORATORY OR BRANCH

3. _____
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. NCI-707C
SERIAL NO

6. EVALUATION OF BLOOD AND PLASMA WATER CONTENT IN PATIENTS WITH MALIGNANT NEOPLASMS
PROJECT TITLE

7. John H. Tuohy
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

The purpose of this project is to determine whether there is a difference in the blood and plasma water content of patients with malignant neoplasms as compared with normal controls and patients with non-malignant diseases. The initiating idea stems from the demonstrated fact that tumor tissue has a high content of water.

Determinations are by the use of the Karl Fischer reagent, a chemical method designed to avoid the tedious method of drying samples to constant weight.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

10. MCI-707C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$9,325	1	—	1	1	—	1	10
FY 1956	$7,225	1	—	1	1	—	1	30

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: None known.

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. NCI	2. GENERAL MEDICINE BRANCH		
INSTITUTE	LABORATORY OR BRANCH		
	4.		5. NCI-708(C)
SECTION OR SERVICE	LOCATION (IF OTHER THAN BETHESDA)		SERIAL NO

SULFHYDRYL AND DISULFIDE METABOLISM IN SKIN AND MUCOUS MEMBRANES AS RELATED TO KERATINIZATION
PROJECT TITLE

Dr. E. J. Van Scott
PRINCIPAL INVESTIGATOR(S)

OTHER INVESTIGATORS

PROJECT DESCRIPTION

PROJECT: This is a continuation of project submitted one year ago under the same title. The continued aim of this project is to further elucidate the role, anatomical and chemical sites of sulfhydryl and disulfide metabolism in the epidermis as occurs in normal and pathological states of the skin.

OBJECTIVE: As above, with the possibility held that significant differences in sulfur metabolism may be found in normal and pathological conditions which will lead to more rational approaches to therapy of certain skin diseases.

METHODS EMPLOYED: Sulfhydryl and disulfide values on lyophilized powdered skin and mucous membrane tissues to be determined, using the sulfhydryl reagent (1-(4-Chloromercuriphenyl-azo)-naphthol-2). More discrete localizations of -SH and S-S will be attempted by isolation of protein fractions of epidermis.

MAJOR FINDINGS: Considerable time has been given to refining the chemical methods used. The previous findings of large amounts of free sulfhydryl in the stratum corneum of psoriatic skin has been reaffirmed, the fact being exploited in a clinical program of therapy for psoriasis.

SIGNIFICANCE TO CANCER RESEARCH: Keratinization is a phenomenon occurring in several types of cancer of skin and other epithelial structures; particularly in squamous cell carcinoma premature keratinization of epithelial cells takes place. By appropriate chemical analyses and examinations of such tumors significant defects in the keratinization process is hoped to be found.

PROPOSED COURSE OF PROJECT: As outlined above but with emphasis placed on examination of more types of tissue specimens.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

10. NCI-708(a) C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 7295	1	2	3	1/2	1/2	1	0
FY 1956	$ 7297	2	2	4	1/2	1/2	1	0

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

15. NCI-7080JC
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

SULFHYDRYL GROUPS AND THE DISULFIDE LINKAGE IN NORMAL AND PATHOLOGICAL KERATINIZATION, VAN SCOTT, E. J. AND FLESCH, P., ARCH. DERM. & SYPH., 1954
70 : 141-154

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

PROJECT REPORT FORM

1. NCI — INSTITUTE
2. GENERAL MEDICINE BRANCH — LABORATORY OR BRANCH
3. — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-7086-C — SERIAL NO

6. HISTOLOGIC AND HISTOCHEMICAL ANATOMY OF CERTAIN SKIN LESIONS — PROJECT TITLE

7. DR. E. J. VAN SCOTT — PRINCIPAL INVESTIGATOR(S)

8. DR. ROSS MAC CARDLE — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

PROJECT: Micro-anatomy of elemental cutaneous structures.

OBJECTIVES: (a) To determine structural changes of tonofibrils in epidermis during the transition of cells to keratinized structures. (b) To determine the elemental changes within the hair follicles which lead to the lesion of acne vulgaris. (c) To study the anatomical changes in epidermis occurring with aging.

METHODS EMPLOYED: Biopsies of human skin are serially sectioned both vertically and parallel to the skin surface and stained with several stains, including H & E, Masson's, Gomori trichrome, Barnett's stain for -SH and S-S. Further sections are micro-incinerated. Reconstruction of the more gross anatomy of hair follicles and other appendages is made by examination of such serial sections.

MAJOR FINDINGS: No statistically verified findings to date.

SIGNIFICANCE TO CANCER RESEARCH: Changes within the epidermis, particularly of the tonofibrils, will be evaluated in the dyskeratatic phenomena seen in skin cancers.

PROPOSED COURSE OF PROJECT: As outlined above, procedures to be carried out on a larger number of skin specimens.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

10. NCI-7088(C)
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$ 11,958	2	1	3	1	½	1½	0
FY 1956	$ 11,934	3	1	4	1	½	1½	0

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]
REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

This project is a cooperative one between the General Medicine Branch of NCI (Van Scott) and the Department of Pathology (MacCardle).

14, IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16 and 17

PROJECT REPORT FORM

1. NCI — INSTITUTE
2. GENERAL MEDICINE BRANCH — LABORATORY OR BRANCH
3. _____ — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-708(C)C — SERIAL NO

6. ARGINASE ACTIVITY OF SKIN AND MUCOUS MEMBRANES AND THE RELATIONSHIP TO NORMAL AND PATHOLOGICAL KERATINIZATION — PROJECT TITLE

7. DR. E. J. VAN SCOTT — PRINCIPAL INVESTIGATOR(S)

8. _____ — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

This is a continuation of project submitted one year ago under the same title, the design of which is to more thoroughly study the enzyme arginase in skin and related tissues.

OBJECTIVES: To determine the arginase activity of epidermal tissues, both normal and abnormal, including carcinoma more thoroughly, and to study the state of activation in which the enzyme is found in the native tissue. Similar studies to be carried out on the "epidermis" of the mucous membranes and tissues undergoing squamous metaplasia.

METHODS EMPLOYED: Epidermis from skin and mucous membranes is separated from underlying corium by the stretch method. Successive layers of human plantar epidermis obtained with microtome. Tissue is dried while in the frozen state and later powdered in a Wiley Mill. "Potential" arginase activity is determined by activation with Mn ion. "Native" activity determined without activation by Mn ion. Determination of arginase activity is done with Dubnoff Shaker and Warburg Respirometer.

MAJOR FINDINGS: Technique of determining arginase has been standardized. Of the tissues studied thus far psoriatic epidermis has been found to be distinctly different from other tissues in that it has a very high potential arginase activity, although having an extremely low native activity.

SIGNIFICANCE TO CANCER RESEARCH: The role and state of arginase is yet to be thoroughly investigated in carcinoma of skin and related tissues for comparative

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

ARGINASE ACTIVITY OF SKIN AND MUCOUS MEMBRANES (cont'd)

purposes, with the possibility held that the activity of the enzyme may be modified in vivo to influence the growth of the tumors.

PROPOSED COURSE OF PROJECT: As outlined above, as well as attempting to isolate the protein fraction of epidermis which contains the enzyme. Isolating of the water soluble proteins will be attempted on protein-separation-columns.

10. WC1-708(c)C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$14,591	1	1	2	1	1	2	0
FY 1956	$14,593	2	1	3	1	1	2	0

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐
REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. NCI
INSTITUTE

2. GENERAL MEDICINE BRANCH
LABORATORY OR BRANCH

3.
SECTION OR SERVICE

4.
LOCATION (IF OTHER THAN BETHESDA)

5. ZCI-7084(C)
SERIAL NO

6. CHEMOTHERAPY OF SKIN CANCER AND SKIN DISEASES BY IONTOPHORESIS
PROJECT TITLE

7. DR. E. J. VAN SCOTT
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

PROJECT: Iontophoresis of materials through the skin.

OBJECTIVES: To determine the effects of galvanic current in skin diseases and skin tumors, and the effect of various substances introduced by iontophoresis.

METHODS EMPLOYED: Suitable chemotherapeutic drugs currently in use, as well as particular metallic ions, are to be introduced into the skin by galvanic current.

PATIENT MATERIAL: (1954 calendar year) None - project now being instituted.

MAJOR FINDINGS: None to date.

SIGNIFICANCE TO CANCER RESEARCH: (a) To determine the direct effect of chemotherapeutic agents on cancers found in the skin; (b) to determine the effect of withdrawal and introduction of metallic ions on skin tumors, hair growth, etc.

PROPOSED COURSE OF PROJECT: As outlined above.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

10. MCI-7086(C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$7,225	1	2	3	1/2	1/2	1	300
FY 1956	$7,227	2	2	4	1/2	1/2	1	300

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM

1. NCI
INSTITUTE

2. GENERAL MEDICINE BRANCH
LABORATORY OR BRANCH

3. _____
SECTION OR SERVICE

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. NCI-708(a)C
SERIAL NO

6. STATISTICAL INCIDENCE AND TYPES OF PIGMENTED LESIONS ON THE PALMS AND SOLES
PROJECT TITLE

7. DR. E. J. VAN SCOTT, DR. CHARLES MC CALL
PRINCIPAL INVESTIGATOR(S)

8. _____
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Pack, on the basis of examinations of 1,000 routine patients, has reported that pigmented lesions occur on the palms and soles in an incidence considerably below 1%. Casual observations of random patients and other individuals by the investigators listed herein would seem to suggest a considerably higher incidence.

OBJECTIVES: To determine statistically the incidence of pigmented lesions occurring on the palms and soles in a randomly selected group of individuals and to determine by biopsy the histological nature of these lesions.

METHODS EMPLOYED: Examination of skin of patients in Clinical Center, as well as employees in Employee Health Unit for palmar and plantar nevi with biopsy-excision of appropriate ones.

MAJOR FINDINGS: To date approximately 300 individuals have been examined and approximately 25% have pigmented lesion on either their palms or soles. Out of seven lesions biopsied, six have been junction nevi histologically.

SIGNIFICANCE TO CANCER RESEARCH: On the basis of Pack's findings that pigmented lesions occur on palms and soles in a negligible incidence and that malignant melanoma occurring on these regions account for approximately 5% of all melanomas, the practice has been to remove all pigmented lesions from these areas. Apart from the above possible erroneous concept regarding incidence of pigmented lesions on these areas, to remove lesions from all large segment of the populations is an impossible undertaking. Fundamentally, though, it would seem imperative to ascertain whether or not pigmented lesions occurring on the palms and soles have any greater predilection toward malignant change than lesions on other parts of the body.

PROPOSED COURSE: Continuation of project as outlined.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

10. NCI-7084-C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$3108	2	—	2	1/3	—	1/3	30
FY 1956	$3092	3	—	3	1/3	—	1/3	60

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

NONE

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NONE

No entries for 15, 16, and 17

PROJECT REPORT FORM

1. Cancer — INSTITUTE
2. Surgery — LABORATORY OR BRANCH
3. _____ — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. ZC1-750 — SERIAL NO

6. Removal of cancer and other tissues for histologic, biochemical or other studies as required by scientists and other investigators for correlated studies. — PROJECT TITLE

7. Dr. R. R. Smith & Dr. J. H. Waite — PRINCIPAL INVESTIGATOR(S)

8. Dr. Donald Cole - Dr. Ross Miller - Dr. Lester Cramer — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

The objective of this project is to supply tissue for histologic, histochemical, biochemical or biophysical studies to scientists working in various branches of N.C.I. who require human tissue for other studies. Usually such studies are done as cooperative projects with various investigators but on occasion specific types of tissue are required by investigators for a specific study, i.e., an investigator has requested that a specimen of skin and its associated neoplasm be made available for studies of keratinization, various muscle biopsies, and/or biopsies of liver tissue under specific conditions have been requested. As a rule these materials are obtained during an operation done for therapeutic reasons.

Methods Employed:

Methods employed are standard surgical procedures.

Patient Material:

The material for this project is largely obtained from patients in for other projects. 2 males and 2 females were admitted specifically for this project, with an average stay of 43 days. No outpatients. 95 operations such as biopsies, excisional biopsies or removal of organs were performed for other branches and/or other institutes.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Major Findings:

Presented in reports of other projects by other investigators.

Significance to Cancer Research:

This project is more or less a service function for other branches of N.C.I. and N.I.H.

Proposed Course of Project:

During the coming year the number of such cases will undoubtedly increase.

PROJECT REPORT FORM (Cont'd)

10. NC1-750C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$ 3,108	1/3	0	1/3	1/3	0	1/3	172
FY 1956	$ 3,092	1/3	0	1/3	1/3	0	1/3	

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

NONE

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NONE

No entries for 15, 16, 17

PROJECT REPORT FORM

1. Cancer — INSTITUTE
2. Surgery — LABORATORY OR BRANCH
4. — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. ZC1-751C — SERIAL NO

Evaluation of Radical Surgery and the Development of New Surgical Techniques as a Therapeutic Means of Palliating or Definitive Therapy of Advanced Cancer.
PROJECT TITLE

Dr. R. R. Smith
PRINCIPAL INVESTIGATOR(S)

Dr. J. H. Waite
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objectives:

Present day cancer therapy is successful only upon complete removal of a cancer or its local destruction by physical agents, i.e., surgery, radiation or chemotherapy. The low over-all salvage rate by any method (15-30%) points to the ineffectiveness of the tools we now use. Examples of this are found in cancer of the cervix and in head and neck cancer. In these two areas the over-all salvage rate is very low. In cancer of the cervix radiation therapy of early Stage I is an effective means of controlling the disease; giving up to 80% five year survivals. However, in Stage III & IV, which is the most common type of cancer seen clinically, the over-all salvage rate drops to from 13 to 0%. The over-all salvage rate for cancer involving the tongue, pharynx, antrum and extrinsic larynx is still under 40%. The total salvage is probably closer to 20%. The reason for the low survival rate, as discussed in the project on "wound seeding", is probably caused by many factors, one of which is the ineffective means we have of taking care of the cancer once it has spread from its primary site. It is the objective of this project to study the cancers of this group from a surgical standpoint, to develop new techniques of therapy, and to allow broadening of the surgical base as an attack upon the group.

Methods Employed:

Patients with advanced cancer of the pelvis and/or head and neck areas, without clinical evidence of widespread dissemination of the disease, have been admitted for complete study. These cases also provided clinical material

for other projects underway in other parts of the Cancer Institute, i.e., metabolic balance observations on advanced cancer, special biochemical studies, endocrine studies, and have provided human cancer tissue for investigators in biochemistry in other areas of the NIH. At the same time they provided material for the surgeons to develop means to help control advanced cancer. This project is in reality composed of a number of projects but because of the relative infrequency of a specific type of cancer, studies must be arranged to take advantage of the clinical material available at any specific time. One of the prominent and interesting facets of this study has been the study of paranasal sinus cancer. As noted in previous reports, cancer of the frontal and/or ethmoid paranasal sinuses is rare. However, when the more common type of antral cancer has extended to any degree, the ethmoid cells are involved and the cribriform area does become involved. An operation has been developed whereby an en bloc resection of the floor of the anterior cranial vault is removed in continuity with the frontal sinus, the ethmoid sinuses, the contents of the orbit, the nose and the maxilla. Several cases of the pharyngeal wall have been obtained for study in which an increasing amount of pharynx has been removed with primary restorative surgery performed.

As noted in the project on virus studies of cervix cancer, 26 cases of cervix cancer have been admitted. In addition, 3 cases with primary carcinoma of the rectosigmoid have been studied in the same manner. The problems involved in uretero-intestinal anastomoses have been investigated and the nine pelvic exenterations in which uretero-intestinal transplants have been performed provided clinical material for observation of these methods. At the present time a method of implanting the ureters is being studied in which a cuff of rectal mucosa is inverted around the nipple of ureter, forming a more natural ostium. The ever-present ascending urinary infection is still present, however, pointing to the ineffectiveness of this means of handling the ureters once extensive surgery of the pelvic organs has been performed.

Patient Material:

As noted in previous projects there is an overlap of patient material between this study and other studies. There are, however, 16 patients who were admitted primarily for this type of study. 12 adult males, 3 adult females, 1 female child - for an average of 56 days. [illegible] Number of Out-patients - 16, Number of Visits - 19.

Major Findings:

During this first year of study the main emphasis has been on the development of technical procedures to allow normal physiologic processes to occur following the radical removal of large portions of the body. There is considerable advancement in the nursing care provided for this type of patient. Procedure for en bloc removal of a large portion of the

paranasal sinus area in continuity with the orbit and part of the cranial cavity has demonstrated the feasibility of this approach and suggests the possibility of more effective initial therapy of paranasal sinus cancer. In the study of pelvic cancer patients undergoing radical surgery methods for developing an effective uretero-intestinal anastomosis have been studied with negative results.

Significance to Cancer Research:

The development of a more effective means of controlling advanced cancers in these areas is a part of the fundamental purpose of the Cancer Institute's program. It also provides a means of obtaining necessary clinical cancer material for other projects in the Cancer Institute.

Proposed Course of Project:

During the coming year it is proposed to continue the study of these cancers which occur at rather infrequent intervals to develop an experience and new methods of primary surgical therapy plus a combination of surgery and chemotherapy to the primary cancers. It is visualized that during the coming years the most fruitful course for such a project to take would be in conjunction with other projects described under the wound seeding experiment whereby definitive therapy of wounds which at the present time are considered only borderline operable will be made curable by means of therapies directed to the wound after excision of the primary tumor.

PROJECT REPORT FORM (Cont'd)

10. NC1-751C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$ 9325	1		1	1		1	892
FY 1956	$ 12,367	1 1/3		1 1/3	1 1/3		1 1/3	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None
NONE

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

N O N E

PROJECT REPORT FORM (Cont'd)

15. NC1-751 C
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Surgical Treatment of Cancer of the Frontal Sinus and Adjacent Areas.

CANCER, Vol. 7, No. 5, September, 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

N O N E

PROJECT REPORT FORM

1. Cancer — INSTITUTE
2. Surgery — LABORATORY OR BRANCH
3. — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-752-C — SERIAL NO

6. Clinical Investigation of the Use of Viral Agents in the Treatment of Human Cervix Cancer. — PROJECT TITLE

7. Dr. Robert R. Smith - Dr. R. J. Huebner — PRINCIPAL INVESTIGATOR(S)

8. Dr. Raymond Christenson — OTHER INVESTIGATORS

9. **PROJECT DESCRIPTION**

Objectives:

To develop means of treating human cervix cancer using virus cultures, attempting to reproduce in vivo the in vitro observation of the destructive action of virus cultures on cells in tissue culture.

Methods Employed:

The laboratory observation that in vitro cultures of APC viruses grown on a tissue culture of human cervix cancer cells caused disintegration of the HeLa cells suggested the possibility of the clinical application of this virus to human cervix cancer. Dr. Huebner and others in NMI are collecting considerable data on the distribution and other characteristics of this group of viral agents. It seems apparent that most of the population during early childhood become infected with one or more types of APC viruses but in the older age groups the antibody level for these viruses becomes very low. If means could be developed to allow the continued growth of such a virus in human cancer, a potent therapeutic weapon would be available.

It had been determined through previous studies involving human volunteers that these virus cultures did not produce any detectable illness when given intranasally. It did not contain any neurotrophic elements, but had been recovered from some cases of acute febrile illnesses in some patients on the NMI wards. The virus is grown on a 5% chick serum in Hanks-Simms solution containing HeLa cells. The culture is filtered through a Sinteroid glass filter to remove all particulate matter before using in this study.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

At the start of the investigation it was decided to try patients with disseminated disease in which all other means of therapy had been exhausted. The first observations were made on patients in which the virus culture fluid was injected directly into the fungating cervical tumor. An additional method of studying the effects of the virus was performed by means of intra-arterial use of the drug. A small polyethylene catheter was passed up the femoral artery to just above the bifurcation of the aorta. With tourniquets applied to both lower extremities, injection of the fluid through the catheter would limit the culture fluid almost entirely to the pelvic structures. To date this procedure has been performed on two patients. Following injection of the virus culture fluid, repeated observations were made of changes in the cervix itself. Repeated biopsies were obtained for both histologic observation as well as cultural studies to determine the presence or absence of virus in the tumor. Later in the study, when the effect of the agent on cervix cancer was a little better understood, a series of four patients were obtained in which the serum of two patients contained antibodies and two did not contain antibodies to the virus used in the study. In 14 to 18 days following the injection of the virus these patients were then subjected to exploratory laparotomy and attempts made to do a total pelvic exenteration or whatever surgery was indicated to remove the cancer. Complete studies on the excised specimens for demonstration of cytologic or bacteriologic changes were then possible. In a more recent study, Cortisone has been used before and during the period of injection of the virus in an attempt to diminish the production of antibodies.

Patient Material:

			Average Stay Days
Admissions:	Adult Male	0	0
	Adult Female	26	68

Outpatients - 26 Visits - 51

Major Findings:

(1) The culture fluid, when injected into a cervix cancer of a patient who has no antibodies to the virus used, obtains a specific cytolytic effect in the area injected. This is characterized by a watery, bloody exudate with the formation of a crater.

(2) When the culture fluid has been heated to a degree insuring that the virus has been killed, the above described effect does not occur.

(3) If a live culture of the virus is injected into the cervix of a patient who has antibodies to the virus used, the above reaction does not occur.

(4) If a second injection of a virus is given to a patient who has previously been sensitive but has recently developed antibodies to the virus, the above described reaction does not occur.

(5) In 10 to 14 days after the injection and after the observable cytolytic action has occurred, there follows a regrowth of the tumor into the crater bed.

(6) In two patients there has been concomitant cavitation of pulmonary metastatic lesions at the time that the viral agent was producing its effect on the local cervical lesion. To date there is no proof that this is other than just a coincident degenerative change in the lung metastases. However, in one of the cases, four metastatic lesions showed the same degree of degenerative changes which would be quite a coincidence.

(7) When the viral agent is given in a susceptible individual, the antibody titer rises promptly suggesting the booster type reaction as seen in routine immunization procedures.

(8) Intra-arterial injection of the viral culture in a patient with antibodies to the virus produced no observable change.

(9) Intra-arterial injection of the virus agent in a susceptible patient did not produce demonstrable disease. It did cause some change in the local cervical lesion which followed somewhat the same pattern of the local injection.

(10) The laboratory studies on cytological changes observed by the pathologists have not been definitely categorized as yet. It has been observed that there are an increased number of mitoses and other changes which require further study to determine the effect on a specific response to the virus.

(11) Laboratory studies to prove the presence of virus in the cervix after the injection have not been completed to date. In one case that came to autopsy several weeks following injection of the virus, the pelvic mass contained extensive degenerative changes extending up as high as the needle had placed the virus culture. This cancer still contained virus.

(12) In a more recent case in which Cortisone was used to inhibit the formation of antibodies, a clinical illness suggestive of a virus infection occurred on the third day, a second peak occurred on the sixth day, and a smaller one on the ninth day, suggesting a continuation of the growth of the virus in the pelvic organs. Laboratory evidence is available to the effect that the virus was present as late as 17 days following injection of the viral agent. In this case, degenerative changes were noted as described above, but there was no appreciable change in the total volume of tumor tissue present in the pelvis.

(13) In nine cases exenterative surgery has been performed for extansive pelvic lesions. Not all of these were on patients in which the virus had been used. Part of them were control cases. One radical hysterectomy was done, and in six patients, in exploring the abdomen, inoperable settings were found which allowed observation of the changes produced above. In one patient, with the abdomen open, the virus was injected directly into the lateral pelvic wall mass over the sciatic notch, which produced equivocable demonstrable results. In most of these cases, biopsy material was obtained

at the time of surgery for histologic and virus studies with almost entirely negative results. In one patient, following exploratory laparotomy where an inoperable situation was found, a colostomy was performed.

Significance to Cancer Research:

This program carries out one of the fundamental aims of the Cancer Institute, that is, of developing new and more effective methods of therapy of common malignant neoplasms. It helps to identify and categorize the newly discovered viruses as far as their reactions on the human tissue is concerned, and may ultimately lead to an understanding of the fundamental nature of cancer itself.

Proposed Course of Project:

During the coming year emphasis will be placed upon expanding the activities to allow more observations of the effectiveness of this type of a procedure. It is hoped that new methods will be developed to supress the antibody production and allow the propagation of the viral agent in the cancer cell. It is recognized that such a therapeutic tool, although it may not be one hundred percent effective in eradicating the body of cancer cells, could cause enough anatomic change in the character of the disease in the pelvis to allow radical surgical removal.

PROJECT REPORT FORM (Cont'd)

10. NC1-752C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$12,433	1⅓			1⅓		1⅓	1764
FY 1956	$21,643	2⅓			2⅓		2⅓	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

N O N E

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

N O N E

No entries for 15, 16, and 17

PROJECT REPORT FORM

1. Cancer — INSTITUTE
2. Surgery — LABORATORY OR BRANCH
4. — SECTION OR SERVICE
LOCATION (IF OTHER THAN BETHESDA)
5. NCI-753C — SERIAL NO

Cytologic determination of the number of tumor cells and/or recurrences recovered or found in an operative wound following removal of a primary cancer in continuity with its regional lymph drainage area containing metastases.

PROJECT TITLE

Dr. R. R. Smith and Dr. Albert W. Hilberg
PRINCIPAL INVESTIGATOR(S)

Dr. Ross MIller
OTHER INVESTIGATORS

PROJECT DESCRIPTION

Objectives:

This project is the clinical part of the over-all study of wound seeding (See Project No.___). Its primary object is to demonstrate the frequency of tumor cells found by cytologic observation when the washings from operative wounds are examined.

Methods Employed:

Patients with locally advanced primary cancers of the head and neck and/or other sites, with regional lymph node metastases, have been admitted for primary surgical therapy. These cases have, for the most part, been those with extensive metastases, i.e., borderline operable cases, for such patients have a greater chance for local recurrence. Following surgical removal of the tumor and its metastases, the open wounds have been washed with saline and the washings studied for the presence of tumor cells.

Patient Material:		No.	Average Stay Days	
Admissions:	Adult males	18	59	
	Adult females	9	70	No. Outpatients - 29
	Children male	1	29	No. visits - 56
	Children female	1	40	

(29 patients - 36 admissions)

Nearly all of these patients were used in other studies and in some patients admitted under other projects wound washings were taken. The 29 patients above, however, were of primary interest in the wound seeding project.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Major Findings:

Wound washings contained demonstrable cancer cells in 8 out of 25 definitive cases. (24%). In cases where tumor cells were found in the washings, two have already developed clinical evidence of recurrent cancer, and in one additional case, melanoma has recurred when the wound washings were negative. The uncontrolled factor, however, in the analysis of such data is that we have no way of knowing if the washing itself is of any therapeutic value.

Significance to Cancer Research:

The demonstration of cancer cells in operative wounds points up the deficiency of a straight surgical approach to cancer therapy. It demonstrates a possible cause of the present low salvage rate in present day treatment of advanced cancer. It is believed that if means were found to prevent this seeding of wounds, the over-all survival rate would be increased 20-40%.

Proposed Course of Project:

To continue with the study as outlined above, -determining the number of operative wounds in which it is possible to demonstrate tumor cells in washings from the operative wound. Once the laboratory part of this study is completed (the study of experimental wound seeding), it is hoped that we will be able to bring to the operating room local wound chemotherapy or other treatment of the open wound as a therapeutic means of preventing recurrences.

PROJECT REPORT FORM (Cont'd)

10. NC1-753c
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$9,325	1	-	1	1		1	1761
FY 1956	$9,275	1		1	1		1	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

NONE

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NONE

No entries for 15, 16, and 17

PROJECT REPORT FORM

1. Cancer — INSTITUTE
2. Surgery — LABORATORY OR BRANCH
3. ____ — SECTION OR SERVICE
4. ____ — LOCATION (IF OTHER THAN BETHESDA)
5. ZMC1-754 — SERIAL NO

6. Evaluation of the Nature, Cause and Means of Preventing Seeding of Operative Wounds with Tumor Cells, using V-2 Carcinoma in Rabbits.
PROJECT TITLE

7. Dr. R. R. Smith
PRINCIPAL INVESTIGATOR(S)

8. Dr. Ross Miller
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To develop methods of producing experimentally in animals multiple recurrent tumors in operative wounds which will allow testing of various therapies aimed at preventing the seeding of wounds.

Methods Employed:

A frequent cause of failure of the surgical treatment of cancer in man is the development of local recurrences in the operative wound. As noted in previous reports, the rate of local recurrences varies considerably. In breast cancer following radical mastectomy, the rate of local recurrence varies between 10 and 20 percent. In Haagensen's clinic where the most strict criteria of operability is followed, the figure still remains high. He reports 12.5% in his personal selected series at Presbyterian Hospital. In head and neck cancers a comparable figure is difficult to obtain because of the fact that the radical surgical en bloc removal of a cancer and its metastases has not been performed for a significantly long period of time to allow collection of statistics. In a personal series of radical removals for advanced head and neck cancers, the local and/or regional recurrence rate was close to 40%. In a recent report from Memorial Hospital in New York on a series of 32 radical head and neck procedures, the over-all salvage was under 40%, and local and regional recurrent cancer was responsible for a large portion of their failures. The same problem holds with gastric cancer as demonstrated by McNeer's figure of 22% local recurrences following gastrectomy for carcinoma of the stomach.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

It is believed that a number of these recurrences are due to seeding of the wounds with cancer cells accidentally placed in the wound at the time of surgery. It is possible that the lymphatics which are transected at the time of surgery contain tumor emboli which, following the trauma and manipulation of the surgical procedure itself, are extruded into the wound. It is also possible that small lymphatics containing tumor cells are left intact at the time of surgery and form a nest for regrowth of the tumor at a later date. If this mechanism of tumor spread can be shown to occur, local chemotherapy of the wound or debridement of the wound of its tumor cells should be an effective therapeutic tool, i.e., a combination of surgical excision of the gross tumor followed by local chemotherapy of the wound, should improve the results of modern day therapy as much as 40%.

In trying to evaluate the clinical effect of wound treatment, and in order to demonstrate the safety as well as the effectiveness of a given chemotherapy or other treatments, it is necessary to develop in the animal a situation which is comparable to that in humans, i.e., to provide a wound which is seeded with tumor. V2 carcinoma in rabbits behaves like an epidermoid carcinoma in man in that it metastasizes to the local lymph nodes and later to the lungs.

The original plans when this project was formulated consisted of trying to reduplicate in the animals the complete surgical situation as seen in man, i.e., to transplant the tumor into a surgically accessible site of an animal and when this tumor developed metastases, do surgical removal of the tumor and its local lymph node metastases, expecting recurrences in a significant number of cases to occur following the surgery. This method has proved impractical for various reasons. Only about 15% of the routine tumor transplants developed local lymph node metastases and 70% or more of the primary implants regressed. It is thus apparent that depending upon the animal to reproduce a seeded wound following surgical removal of a transplanted tumor would not produce a statistically significant number of cases to allow evaluation of the effectiveness of any therapeutic measure. To overcome this we are now studying the possibility of omitting the first step of the procedure and are now attempting to seed wounds directly by contamination of open surgical wounds. One of the two wounds on each animal is then subjected to the therapeutic procedure. In a more recent experiment in which we attempted direct seeding of operative wounds, 6 out of 7 animals have developed palpable nodules in operative wounds. Postoperative washing with saline of one of the parallel wounds on each animal did not change the development of nodules in 5 of the 6 animals.

Major Findings:

During the past year most available time has been spent on learning the characteristics of V2 carcinoma as applied by our techniques. The problem of maintaining a standard inoculum has not been solved. Metastases have not developed as frequently as had been anticipated, i.e., of 44 animals that died of their tumors, 17 had local regional metastases, 12 had lung metastases, and in three there was widespread disseminated cancer.

In general, the tumor in our hands has been characterized as follows:

(a) For several days, within limits, the size of inoculum does not affect the growth rate, and tumor size is nearly proportioned to inoculum size.

(b) At about 14 days, growth slows or stops, then either resumes at a slower rate, or the tumor regresses completely. This response depends upon the size which the tumor has attained by this time.

(c) We have not been able to compare early growth rates between intramuscular and subcutaneous tumor, but intramuscular tumors maintain their growth with much less of a break at 14 days than subcutaneous ones. At any particular inoculum size, intramuscular inoculation will give a higher percentage of tumors which eventually become large and kill the host. With a good start, such tumors will grow rapidly and kill in three waeks.

(d) Subcutaneous tumors, if planted at the same time in the same animal with intramuscular implants, show much less of a break in their growth curves at 14 days. Apparently the large, vigorous intramuscular tumor overcomes the host resistance in some systemic fashion, possibly by a hyper-immunity mechanism.

(e) Tumors arising from very small inocula, such as occur in needle punctures or wound swabbing, reach a palpable size in about 12 days, but being very small, would then regress unless there is some special device to prevent this, such as a large intramuscular tumor elsewhere.

We have been using New Zealand white rabbits because of their ready availability, but we have occasionally used Dutch and chinchilla rabbits. We have no reason to suppose that this immunity mechanism would be any less inconvenient in these other breeds.

Significance to Cancer Research:

If it becomes possible to reproduce in the animal the conditions found in human cancer patients, i.e., recurrent tumor dissemination, it will then be possible to test experimentally various therapies when applied to wounds. This would allow the development of effective local chemotherapy and improve the over-all salvage from present day methods of cancer treatments.

Proposed Course of Project:

(1) To further establish methods of producing experimentally seeded wounds.

(2) To develop a more uniform inoculum so that there will be less variability from one experiment to another.

(3) To treat these wounds with various drugs, enzymes or physical agents in an effort to prevent wound seeding.

PROJECT REPORT FORM (Cont'd)

10. NC1-754
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$3108	1/3	0	1/3	1/3	0	1/3	
FY 1956	$6183	2/3	0	2/3	2/3	0	2/3	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

N O N E

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

N O N E

No entries for 15, 16, and 17

PROJECT REPORT FORM

1. Cancer — INSTITUTE
2. Surgery Branch — LABORATORY OR BRANCH
3. _____ — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-7556 — SERIAL NO.

6. Development of new methods for the treatment of lung cancer, using regional chemotherapy: Clinical Research. — PROJECT TITLE

7. Dr. J. H. Waite & Dr. Robert Sullivan — PRINCIPAL INVESTIGATOR(S)

8. Dr. A. G. Morrow (Thoracic Surgeon) & Dr. Wm. Banfield (Pathologist) — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project:

Development of new methods for the treatment of lung cancer, using regional chemotherapy: Clinical Research.

Objectives:

The objective of this experiment is to develop a method for regional chemotherapy of advanced primary and metastatic neoplasms of bronchi and lungs. The methods developed by animal research under Project No. ___ will be applied as evolved.

Methods Employed:

Patients admitted with apparently operable primary and solitary metastatic carcinomas of the lung are subjected to thoracotomy. If thoracotomy discloses previously undiagnosed inoperability (and this will occur in about 75%), the tumor is biopsied. The end of a small plastic catheter is implanted in a peripheral radical of a pulmonary vessel, and the catheter's other end exteriorized through the thoracic wall. Respiratory dynamics are studied postoperatively by measurement of intravascular pressures and gas saturations via the catheter.

Chemotherapeutic agents are injected through the catheter into the regional blood supply to the involved lung. Serial measurements of the size of the tumor shadow are made. Changes, if observed, are compared with changes in other untreated portions of the lung. The patient is then carefully followed, and in event of demise, careful autopsy study is performed to compare the treated lung tissue with previous operative findings for drug effect. Nitrogen mustard

is being used as the standard drug until a standard technique of administration is developed.

Patient Material:		No.	Average Stay Days
Admissions:	Adult males	1	30
	Adult females	3	30
Follow-up visits:		9	

Major Findings:

Project underway only one month. Of the four patients studied, two had resectable lesions. In the other two, catheters were implanted successfully and postoperative studies performed. No untoward effects of the catheterization occurred. No final results are available.

Significance to Cancer Research:

Continuing increase in the incidence and death rate due to lung cancer demands maximal efforts to control the disease. The primary or basic science approach involves efforts to determine the biologic nature and environmental relationships of lung cancer. While of great hope, this research has yet to produce applicable results. A second phase is seen in programs aimed at the discovery and treatment of lung cancer in its earliest stages. This attack has succeeded in curing up to 5% of all patients seen by extirpative surgery, the only curative treatment now known. The third phase of lung cancer research involves improvement of treatment of the disease when it is past its earliest stages, and the large number of well-advanced lung cancer patients now seen provides an urgent motivation for the clinical investigator to search for an approach such as described in this project.

Proposed Course of Project:

The continued study of catheter implantation and drug administration methods is planned, utilizing methods as developed in laboratory Project No.__. Nitrogen mustard will be continued to be used as the standard drug until administration techniques are standardized. Then other possibly chemotherapeutic agents will be considered, using the nitrogen mustard effect for comparison.

10. NCI-755C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	0	0		0	0		0	120
FY 1956	$3,092	1/3		1/3	1/3		1/3	

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

N O N E

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

N O N E

No entries for 15, 16, and 17

PROJECT REPORT FORM

1. Cancer — INSTITUTE
2. Surgery Branch — LABORATORY OR BRANCH
3. — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. ZA-756 — SERIAL NO.

6. Development of new methods for the treatment of lung cancer, using regional chemotherapy: Animal Research — PROJECT TITLE

7. Dr. J. H. Waite & Dr. Robert Sullivan — PRINCIPAL INVESTIGATOR(S)

8. Dr. William Banfield, Pathologist — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project:

Development of new methods for the treatment of lung cancer, using regional chemotherapy: Animal Research.

Objective:

The objective of this experiment is to develop a method for regional chemotherapy of advanced primary and metastatic neoplasms of bronchi and lungs. It will be attempted to produce a high level dosage to lung cancer tissues, with minimum damage to the rest of the lung, to the hematopoetic organs, and the rest of the body. As developed, methods are applied to human cases of lung cancer under project no. ___.

Methods Employed:

Using normal laboratory dogs as research subjects, small polyethylene catheters are implanted into the lumen of a bronchial artery, a pulmonary artery, or a pulmonary vein, and the other end of the catheter exteriorized for studies of pulmonary hemodynamics and injection of drugs. Pulmonary hemodynamics are studied by pressure measurements, blood sample gas studies, and angiograms using injected radiopaque dye. The initial study drug is nitrogen mustard. Morphologic changes in bronchi, lymph nodes and pulmonary tissues are studied after injecting the drug through one of the three enumerated vascular routes.

Methods for potentiating drug effect on bronchi and lymph nodes while decreasing drug effect on alveoli are being studied in accordance with pathologic studies by Wood and Miller of Stanford University and by others, which have shown that lung tumors derive their major blood supply via the bronchial blood vessels.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Major Findings:

Work on this project has consisted of preliminary studies for the development of techniques and one series of dogs treated by intra-pulmonaryvenous nitrogen mustard after preparation by permanent ligation of pulmonary lobar vein with or without simultaneous ligation of the pulmonary lobar artery. Pathologic studies on the series are underway, and no definitive findings are yet available. Results to date suggest that injection via the bronchial artery produces the most specific local effects on the hilar portions of the bronchial tree.

Significance to Cancer Research:

Continuing increase in the incidence and death rate due to lung cancer demands maximal efforts to control the disease. The primary or basic science approach involves efforts to determine the biologic nature and environmental relationships of lung cancer. While of great hope, this research has yet to produce applicable results. A second phase is seen in programs aimed at the discovery and treatment of lung cancer in its earliest stages. This attack has succeeded in curing up to 5% of all patients seen by extirpative surgery, the only curative treatment now known. The third phase of lung cancer research involves improvement of treatment of the disease when it is past its earliest stages, and the large number of well-advanced lung cancer patients now seen provides an urgent motivation for the laboratory investigator to search for an approach such as described in this project.

Proposed Course of Project:

Continued studies will be made to determine the most advantageous pulmonary vascular route of drug administration, and to attempt to potentiate the drug action by permanent or temporary diversion of the blood stream. After developing the method, nitrogen mustard dosages required to produce effects on regional bronchial and lymphatic structures will be studied, and correlated with the degree of hematopoetic depression. Concurrent application of methods developed to advanced human lung cancers is planned.

PROJECT REPORT FORM (Cont'd)

10. NCI-756
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	0	0		0	0		0	0
FY 1956	$3,092	1/3		1/3	1/3		1/3	

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]
REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

N O N E

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

N O N E

No entries for 15, 16, and 17

PROJECT REPORT FORM

1. Cancer — INSTITUTE
2. Surgery Branch — LABORATORY OR BRANCH
3. — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. Z01-157-C — SERIAL NO

6. Prevention of operative site recurrence by biopsy wound closure with plastic spray: Clinical Research. — PROJECT TITLE

7. Dr. J. H. Waite — PRINCIPAL INVESTIGATOR(S)

8. Dr. R.R. Smith & Dr. A. W. Hilberg — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project:

Prevention of operative site recurrence by biopsy wound closure with plastic spray: Clinical Research.

Objectives:

To develop a new improved method for biopsy site closure applying results of the laboratory research Project No.___.

Methods Employed:

Patients with apparently primary operable breast carcinoma will be admitted. A few patients should supply the necessary significant information. Local excision, examination and frozen section will be performed to confirm the diagnosis. The biopsy site will be closed in the usual manner. Then a geometric half of the biopsy wound skin surface will be masked off, and cell washings for cytologic study taken from the remaining portion of the skin surface. The entire wound will then be sealed with a plastic spray, using the method as developed in the laboratory project. Cell washings for cytologic study will then be taken from the originally masked geometric half of the skin surface in the operative field, and pre- and post-treatment washings compared for presence and quantity of exfoliated tumor cells.

Major Findings:

None. Project not started.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Significance to Cancer Research:

Wound recurrence following biopsy and local surgery is not uncommon. In treating one of the most common cancers, that of the breast, Massachusetts Memorial Hospital surgeons admit to 11% and Haagensen reports 12% local recurrences. The two most likely factors are inadequate surgical margins and spillage of cells at time of biopsy. Preliminary investigations by Dr. R. R. Smith and by the principal investigator have recovered exfoliated cells from skin surfaces of clinical cases, even after more than usually careful attempts at biopsy site closure and sealing off, using conventional technique. A simple method which would guarantee 100% riddance of the skin surface tumor cells would be of immediate value to all surgeons in treatment of many common malignancies.

Proposed Course of Project:

The entire project is now proposed. If successful, the method will be recommended to surgeons for clinical application.

10. NO1-757C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	0	0	0	0	0	0	0	0
FY 1956	$4638	3	0	3	1/2	0	1/2	0

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

none

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

none

no entries for 15, 16, and 17

PROJECT REPORT FORM

1. Cancer — INSTITUTE
2. Surgery Branch — LABORATORY OR BRANCH
3. — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. ZCl-758 — SERIAL NO

6. Prevention of operative site recurrence by biopsy wound closure with plastic spray: Laboratory Research. — PROJECT TITLE

7. Dr. J. H. Waite — PRINCIPAL INVESTIGATOR(S)

8. Dr. R. R. Smith & Dr. A. W. Hilberg — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project:
Prevention of operative site recurrence by biopsy wound closure with plastic spray: Laboratory Research.

Objectives:
To develop by animal experiment a method for reducing operative site recurrence after local biopsy in radical surgery for cancer.

Methods Employed:
Using an appropriate small animal/tumor, a skin incision and biopsy will be made and the biopsy wound closed by the usual method. One half of the animals will be treated additionally by spraying the biopsy site with a liquid plastic surgical dressing material. Washings from the wounds will be examined by cytologic methods for presence and number of tumor cells.

Major Findings:
None. Project not started.

Significance to Cancer Research:
Wound recurrence following biopsy and local surgery is not uncommon and treating one of the most common cancers, that of the breast, Massachusetts Memorial Hospital surgeons admit to 11% and Haagensen reports 12% local recurrences. The two most likely factors are inadequate surgical margins and spillage of cells at the time of biopsy. Preliminary investigations by Dr. R. R. Smith, and by the principal investigator, have recovered exfoliated cells from skin surfaces of clinical cases, even after more than usually careful attempts at biopsy site closure and sealing off using

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

conventional technique. A simple method which would guarantee 100% riddance of the skin surface tumor cells would be of immediate value to all surgeons in treatment of many common malignancies.

Proposed Course of Project:

The entire project is at present in the proposed status. A project involving clinical application is planned, if warranted, by results in the animal project.

PROJECT REPORT FORM (Cont'd)

10. NCI-708
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$7,295	3	1	4	1/2	1/2	1	0
FY 1956	$7,297	3	1	4	1/2	1/2	1	0

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

NONE

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NONE

No entries for 15, 16, and 17

PROJECT REPORT FORM

1. Cancer — INSTITUTE
2. Surgery Branch — LABORATORY OR BRANCH
3. ____ — SECTION OR SERVICE
4. ____ — LOCATION (IF OTHER THAN BETHESDA)
5. NC 1-759 — SERIAL NO

6. Development of a technique for performing a successful hypophysectomy in the guinea pig. — PROJECT TITLE

7. Dr. Lester Cramer & Dr. Eli Nadel — PRINCIPAL INVESTIGATOR(S)

8. None — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project:
Development of a technique for performing a successful hypophysectomy in the guinea pig.

Objective:
The objective of this project is to hypophysectomize guinea pigs and keep them alive for at least thirty days postoperatively.

Methods Employed:
The various approaches to the pituitary that have been successful in other species will be tried on the guinea pigs, and postoperative replacement therapy will be investigated.

Major Findings:
None. Project not under way as yet.

Significance to Cancer Research:
The relationship of the endocrine system to tumor tissue is well established. It has not been completely nor thoroughly evaluated in the guinea pig. One major reason for this deficiency has been the inability to use an hypophysectomized guinea pig for study because the animals die too early in the postoperative course.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Proposed Course of Project:

The failures of other investigators seem related to both technique and to postoperative management. The parapharyngeal, transbuccal, transorbital, and transtemporal approaches to the pituitary gland will be evaluated, and the use of dietary and hormonal supplements will be evaluated in the postoperative course.

When a successful preparation has been achieved, then these animals could be used in the study of the pituitary mediation of hormonal effects upon tumors and other tissue.

10. NC1-759
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	0	0		0	0		0	0
FY 1956	$3,092	1/3		1/3	1/3		1/3	0

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

NONE

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NONE

No entries for 15, 16, and 17

PROJECT REPORT FORM

1. Cancer — INSTITUTE
2. Surgery — LABORATORY OR BRANCH
3. — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. ZC1-760 — SERIAL NO

6. A study of the effects of Beta-naphthylamine and 4-Aminodiphenyl on gastrointestinal and bladder mucosa by direct contact in dogs.

7. (1) Development of a surgical technique for in vivo study of drugs in the gastrointestinal tract and the isolated urinary bladder; (2) A study of the techniques for handling the genitourinary tract after cystectomy.
PROJECT TITLE

8. Dr. Donald Cole - Dr. W. C. Hueper
PRINCIPAL INVESTIGATOR(S)

9. —
OTHER INVESTIGATORS

10. PROJECT DESCRIPTION

Objectives:

(a) Development of an experimental method by which, through the local application of certain aromatic amines and related compounds to the mucosa of the gastrointestinal tract and bladder, isolated from its normal connections and the influence of the gastrointestinal content and urine, respectively, cancerous responses can more readily be attained than by administering the test agents by mouth, thereby causing a marked dilution of the chemical at the point of contact.

(b) Development of a method permitting the study of metabolites of aromatic amines and their derivatives in different parts of the gastrointestinal tract.

(c) Development of a surgical technique with minimal hazards for handling the genitourinary tract after cystectomy.

Methods Employed:

(a) An isolated loop of colon is prepared in a normal dog and implanted into the abdominal wall so that carcinogens can be directly applied to the isolated mucosa and anatomic and metabolic changes occurring can be observed through direct observation, biopsy and chemical analysis of secretions. Similar studies are undertaken with intestinal loops from various parts of the small intestine and duodenum with and without bile and pancreatic juice.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

(b) All the current methods for handling the upper genitourinary tract after cystectomy have been investigated. An attempt is being made to develop a surgical technique which will minimize or do away with the genitourinary complications that are a frequent aftermath of the techniques usually employed.

Major Findings:

The major findings of this project thus far are the preparation of the dogs now ready for the application of carcinogens. Thus far the Mathieson technique for uretero-sigmoidostomy appears to be the best one employed.

Significance to Cancer Research:

Previous experimental work with rats and dogs has shown that certain aromatic amines and azocompounds produce cancers of the bladder, liver and intestinal mucosa. While it is established that the aromatic amino compounds, beta-naphthylamine and benzidine, elicit cancers of the bladder in man, similar evidence is not available for 4-aminodiphenyl and a number of aromatic azocompounds which contain the two aromatic amines as a constituent group; nor are human and experimental data on hand whether these and other aromatic aminocompounds, especially dyes and anti-oxidents, exert carcinogenic effects upon the gastrointestinal mucosa.

For this work it was necessary to develop a method for studying these compounds on isolated segments of the gastrointestinal tract and the isolated bladder. The latter has further application in the fact that none of the techniques necessary after cystectomy for handling the upper genitourinary tract are very satisfactory.

Proposed Course of Project:

The proposed course of this project is an attempt to find the answers to questions proposed in the previous paragraph during the next year.

PROJECT REPORT FORM (Cont'd)

10. NC1-760
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	0	0 1/3		1/3	0	0	0	none.
FY 1956	$ 3,092	1/3		1/3	1/3		1/3	

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

NONE

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NONE

No entries for 15, 16, and 17

PROJECT REPORT FORM

1. Cancer — INSTITUTE
2. Surgery Branch — LABORATORY OR BRANCH
3. — SECTION OR SERVICE
4. Memorial Hospital, N.Y.C. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-761 — SERIAL NO

6. Blood-Pressure Servo-Regulator — PROJECT TITLE

7. Dr. J. H. Waite — PRINCIPAL INVESTIGATOR(S)
   John Laughlin - Physics Dept., Sloan Kettering Institute, New York City
   Naomi Sager " " "
8. William Poppell - Physiology Lab. " "
   ~~William Dovland - Anesthesia Dept., Memorial Hospital~~ " — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project:

Blood-Pressure Servo-Regulator

Objective:

To construct a machine for automatic regulation of blood pressure in shock due to vasodilation, and in hypotensive anesthesia.

Methods Employed:

The mean arterial blood pressure is measured directly with a transducing manometer. Appropriate vasodepressor (norepinephrine) or vasodepressor (arphonad) solution is administered continuously intravenously by a variable speed infusion pump. The pump speed is regulated by a signal generated by a servo-loop. The servo-loop signal is proportional to the difference between the blood pressure measured, and the blood pressure clinically desirable for the patient being treated. The therapist thus needs only to set the control panel for the desired blood pressure, and the auto-regulated machinery administers blood pressure regulating substance as needed.

Major Findings:

Work on this project during the past year has consisted of design and construction of the machine. Entire work on the project has been carried on at the Sloan Kettering Institute under the direction of the principal investigator. The design has been completed and the construction is near completion.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Significance to Cancer Research:

The machine, if successful, will be an aid to maintaining blood pressure in patients with neurogenic shock due to radical surgery or drug reactions. It will also provide a method for controlled lowering of blood pressure (hypotensive anesthesia) in patients undergoing radical surgery for cancer.

Proposed Course of Project:

During the coming year it is planned to complete construction of the machine, carry out laboratory testing on animals, and to apply it to appropriate human cancer cases at Memorial Hospital.

PROJECT REPORT FORM (Cont'd)

10. NCJ-761
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$4,662	0	—	6	½	0	½	
FY 1956	0	0	0	0	0	0	0	0

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

N O N E

No entries for 15, 16, and 17

PROJECT I

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Endocrinology Branch — LABORATORY OR BRANCH
3. Endocrinology Service — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. ZC1-800C — SERIAL NO

6. Endocrine Aspects of the Progression and Therapy of Cancer of the Breast in Women and Men — PROJECT TITLE

7. Roy Hertz — PRINCIPAL INVESTIGATOR(S)

8. Max G. Sherer, James Pittman, A. Breslow, H. Altman and L. DeGroot — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION A) Objectives: 1. To extend and intensify detailed clinical observation of the natural course of cancer of the breast in men and women in order to better appraise the effectiveness of various methods of therapy and learn more concerning the etiology and pathogenesis of the disease. 2. To improve upon existing forms of hormonal therapy for palliation of advanced breast cancer and to elucidate the mechanisms involved.

B) Methods employed: 1. The acceptance of complete clinical responsibility for the further management of referred patients with proven diagnosis of cancer of the breast and the application to their problems of all available modes of therapy. 2. The exploration of new modes of hormonal therapy for palliation. 3. The detailed analysis of endocrine and metabolic factors operating in relation to the genesis and course of the disease in these patients up to the time of their demise. 4. Careful appraisal of pathological specimens obtained at biopsy or autopsy in collaboration with Pathology staff.

C) Major findings: (1/1/54-12/1/54). 1. Cooperation with Committee on Therapeutic Trials of the American Medical Association has continued. A total of approximately 170 cases of breast cancer has now been studied with varying degrees of intensity and for periods ranging from 3 months to 5 years. This experience has provided a valuable clinical background for the evaluation of various therapeutic attempts and for the familiarization of our personnel with the clinical manifestations of breast cancer in various age groups. This activity has also effected the necessary professional liaison with local physicians and medical institutions for the further referral of patients to the Clinical Center for these studies.

(see attached sheet)

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Attachment 1. C) (continued)

2. The clinical feasibility of massive parenteral estrogen therapy of breast cancer has been established by these studies. The applicability of water-soluble estrogens by either intravenous or subcutaneous routes has been determined. Methods for the suppression of occasional toxic reactions by providing a salt free diet liberally supplemented by vitamins of the B-complex have been developed. Clinical therapeutic results obtained by intensification of estrogen therapy have indicated that more profound and more rapid regressions of breast cancer can be obtained in this manner. Much more extended experience will be necessary in order to assess the precise degree of advantage which this approach provides over results which can be obtained by more conventional methods. Such evaluation is a long-term problem but initial observations encourage further study of this method.

3. The feasibility of massive intravenous infusion of estrogen has permitted the development of a number of studies of estrogen metabolism in varying clinical states. Bio-assay methods for serum estrogen applied to the serum of estrogen-treated patients with breast cancer have indicated that the estrogen is rapidly cleared ffom the blood stream. Composite clearance data from 20 cases have permitted us to draw an estrogen tolerance curve which may now be used as a reference curve for the determination of hepatic, biliary, and renal factors involved in estrogen clearance from the body.

4. Assay of pleural fluid and ascitic fluid from patients getting massive estrogen dosages intravenously has shown that this hormone equilibrates between the patients' plasma and these transudates. This suggests that the occasional beneficial effect on fluid accumulation in the pleural or abdominal cavity may be a direct effect on the local metastases rather than a systemic metabolic effect.

5. Further observations have extended our earlier data on the quantitative interference with biliary excretion of bromsulphonphthalene by the maintenance of a high blood level of estrogen. This may provide a tool for the further study of the hepatic metabolism of estrogen and for the prolongation of the hepatic destruction of a given dose of estrogen.

6. Studies on the renal clearance of intrapleurally administered phenolsulphonphthaleine (P.S.P.) in patients with hydrothorax have shown that estrogen has no regularly reproduceable effect on such clearance. This indicates that estrogen produces its effect on pleural effusion in some manner other than producing a direct effect on membrane permeability.

7. Although it is clearly established that estrogen withdrawal bleeding can be uniformly produced in women under 40, our data show that only 15 per cent of our older patients with breast cancer (60 years old and over) show withdrawal bleeding when estrogens are stopped. This suggests the necessity for the presence of another factor which is present in younger women for endometrial response to exogenous estrogen. This is presumably an extra gonadal factor and warrants further study.

8. All of our patients with breast cancer on prolonged estrogen therapy have been carefully followed for the possible occurence of neoplastic change in the cervix or endometrium. No such changes were noted in Papanicolaou smears or in authpsy specimens. Two patients, who are still living, developed atypical endometrial hyperplasia which did not regress after estrogen cessation. Whether these patients will ultimately develop fundal carcinoma remains to be seen. Our

Attachment 2. C) (continued)

observations indicate the comparative lack of carcinogenicity of estrogens in the female genital tract even after prolonged therapy with massive dosage.

9. Initial studies with massive intravenous androgen administration have been undertaken in younger patients with breast cancer in whom the dominant problem is that of bone pain. These efforts are just starting and thus far we can only say that the procedure is well tolerated under controlled hospital conditions. Parallel studies to those carried out on estrogen regarding the metabolism of androgens are stymied for the moment since we have thus far been unable to demonstrate by bioassay only active androgen in the blood of patients receiving massive dosages. However, further analysis by chemical means may resolve this difficulty for us.

10. We have initiated a study on the effect of the protein anabolic action of androgen upon the patient's urinary clearance of dietary trace factors including riboflavin, biotin, niacin, and pantothenate. This has involved the development of balance techniques employing a paired-tray procedure. After surmounting considerable methodological difficulties we have collected some initial data indicating that whereas the protein anabolic effect of androgen is accompanied by no material change in urinary balance of biotin, riboflavin, niacin or folocin, it is associated with pantothenate retention. The significance of this association is as yet unclear but may help to clarify the mechanism of the protein anabolic effect of androgens, and effect which is found to be so valuable in the supportive therapy of advanced cancer. (See project VI).

D) Significance to the Program of the Institute: The direct pertinence of the foregoing studies to the grave problem of advanced or persistent breast cancer is too obvious to require discussion.

E) Proposed Course of Project: 1. Continuation and extension of the foregoing studies is proposed. With superior facilities now available, we expect to better index and codify the clinical data as they accumulate and this is being worked out with the Medical Records Dept. of the Clinical Center. It may be possible to have all the records of our previous cases similarly indexed and codified but this may prove impracticable.

2. Further assessment of the place of massive estrogen and androgen therapy in palliation of advanced breast cancer is projected.

3. A correlation of the nature of the estrogen tolerance curve and other aspects of hepatic and renal function is to be carried out.

4. An extended study of the effect of various steroids on the dynamics of fluid accumulation in the pleural cavity and in the abdomen is planned.

5. The quantitative data on hepatic blockade by estrogens are to be extended.

6. A collaborative study with Drs. Hilberg and Thomas has been set up for the more complete evaluation of the histological and cytological effects of steroids on the breast cancer as well as upon the tissues of the female genital tract. The

Attachment 3. E) (continued)

reason for the relative refractoriness of the endometrium of the senile patient will be given special attention. The detection of any malignant or premalignant changes in the tissues of the female genital tract will be emphasized.

7. A detailed study of the metabolic and endocrine effect of preoperative treatment of premenopausal women undergoing surgical ovariectomy with potent gonadotropic hormone preparations. This will provide an opportunity to evaluate the exact morphological effects of various gonadotropins on the human ovary.

Studies on the mechanism of hormone-induced hypercalcemia in patients with advanced breast cancer are to be initiated this year.

8. Studies on the metabolism and fate of intravenous androgen will be extended with emphasis upon the acute effects on bone pain and upon the balance of nitrogen, electrolytes calcium and dietary trace factors and upon the clearance of the androgen itself.

9. Clinical trials will be made with other steroid substances including progesterone, 19-norprogesterone, cortisone, and hydrocortisone separately and in varying combinations with estrogen and androgen. These studies will be appropriately supplemented by metabolic and histological observations of the effects of these potent compounds upon the breast cancer and upon the female genital tract.

10. Further search (correlated with projectIX) for compounds lacking estrogenic, androgenic, or corticoid effects and yet capable of suppressing pituitary gonadotropic and adrenotropic action will be conducted. Further screening for protein anabolic compounds lacking androgenic action will be likewise pursued and such compounds will be assessed clinically.

PROJECT REPORT FORM (Cont'd)

PROJECT I

10. NCI-8CCC
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$27,975	6	0	6	3	0	3	1200
FY 1956	$27,825	6	0	6	3	0	3	1200

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT I

15. NCI-800C
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Much of the foregoing work is of such long term that publication will be deferred for at least 1 more year.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954: Hertz, Roy: a) reelected Chairman, Panel on Endocrinology, Committee on Growth of the National Research Council and member Executive Committee of C.O.G.
b) Served on faculty of John Andrews Clinical Society, Tuskegee, Alabama, April 11-16, 1954.
c) Elected as Director of the American Association for Cancer Research for period 1954-57.
d) Invited to present exhibit at International Conference of Obstetrics and Gynecology, Geneva, Switzerland, August 1954 (not attended).

PROJECT REPORT FORM

PROJECT II

1. National Cancer Institute — INSTITUTE
2. Endocrinology Branch — LABORATORY OR BRANCH
3. Endocrinology Service — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-80/C — SERIAL NO

6. Endocrine Aspects of Cancer of the Prostate in Man
PROJECT TITLE

7. Roy Hertz and Lois F. Hallman
PRINCIPAL INVESTIGATOR(S)

8. James Pittman, Harold Altman, Leslie DeGroot and Alexander Breslow
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION A) Objectives: 1. To improve non-surgical therapy of cancer of the prostate.
2. To increase understanding of endocrine factors involved in genesis and course of prostate cancer.
3. To gain basic knowledge of the metabolic action of certain steroids in man.

B) The proposed Studies include: 1. Extension of clinical evaluation of massive intravenous estrogen therapy in cases of advanced prostate cancer no longer amenable to customary anti-androgenic therapy indicate that further clinical remission can be obtained by bombarding these patients with estrogen dosages previously considered to be acutely toxic. Blood level data indicate that levels can be obtained in this manner which are unattainable by other routes of administration. Although the initial observations are highly encouraging, so many variables enter the clinical picture that a final evaluation will require systematic observation of 50 to 100 cases by a group of experienced observers. Quantitative data on the weight, food intake, hemoglobin, plasma proteins, serum acid and alkaline phosphatase, narcotic requirement, ambulation, and work output are to be tabulated. The radiological appearance of osseous metastases is to be followed by periodic x-rays. Periodic biopsies of available primary or metastatic cancer are to be renewed for evidence of regression. Urethrograms and barium enemata, as well as digital evaluation will be used to record change in size of the local tissue mass in appropriate cases.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Attachment 1 B) (con't.) PROJECT II

2. Preliminary observation on the lack of acute effect of high estrogen blood level on serum acid phosphatase level will be extended by combined bioassay and biochemical study of the plasma.

The nature of the acid phosphatase activity and the effect of the therapy on the respective "prostatic" and "non-prostatic" phosphatase fractions will be followed as a special study by Miss Lois Hallman.

The problem of the mechanism of elevation of acid phosphatase in metastatic prostate cancer will be approached in the light of our published canine experiments which indicate that some factor other than mere ectopic location of the active tissue is essential for the entrance of the enzyme into the blood stream. (Correlate with Project IX). Preliminary data indicate the existence of a normal inhibitory substance which is lacking in the plasma of patients with a high acid phosphatase titre. This problem is to be pursued further.

3. Attempts to ascertain the half-life of acid phosphatase in the peripheral circulation will be made by determining arterio-venous differences in the titre in vessels of involved parts as compared with vessels supplying uninvolved portions of the body and by applying tourniquets for varying periods of time.

4. Medical adrenalectomy will be attempted by (a) administration of massive infussions of hydrocortisone properly covered by adequate potassium dosage and salt restriction plus excess protein hydrolysate to cover the catabolic effect, thus avoiding acute toxicity from the glucocorticoid itself; and

Administration of amphenone and related compounds under conditions which will permit evaluation of their effect upon adrenal androgenic function.

C) Significance to the Program of the Institute: The high frequency of prostatic carcinoma in man and initial inroads upon it by hormonal therapy indicate the pertinence of this study to cancer research.

PROJECT REPORT FORM (Cont'd)

PROJECT II

10. NCI-801C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	0	0	0	0	0	0	0	0
FY 1956	$ 23,868	6	1	7	2	1	3	600

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT II

15. NCI-801C
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

None. New projedt; preliminary data on hand do not warrant publication thus far.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

PROJECT REPORT FORM

PROJECT III

1. National Cancer Institute (INSTITUTE) 2. Endocrinology Branch (LABORATORY OR BRANCH)

3. Endocrinology Service (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. ZCI-807 C (SERIAL NO)

6. Preoperative Hormonal Therapy of Cancer of Cervix
PROJECT TITLE

7. Roy Hertz and Robert Smith
PRINCIPAL INVESTIGATOR(S)

8. James Pittman, Harold Altman, Leslie DeGroot and Alexander Breslow
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION A) Objectives: To determine the value of steroid therapy as a palliative measure in advanced cancer of the cervix.

B) Methods: Patients who are considered amenable to definitive surgical therapy are pre-treated for 4 to 6 weeks with massive doses of various steroid hormones singly and in combination and then are operated upon. Their progress is evaluated by frequent pelvic examination, serial biopsies and smears and by histopathological study of the surgical specimens removed. Pyelograms and barium enemata are also employed to evaluate changes in the pelvic viscera. The amount of vaginal bleeding is checked from day to day. The patient's general clinical status with respect to weight, appetite, blood status, and pain are also closely observed.

C) Major findings: Initial observations in 28 cases of cervical cancer treated with progesterone revealed minor changes which were erroneously interpreted to reflect a regressive change in the lesions. However, further familiarity with the spontaneous fluctuation in the gross appearance of such lesions leads us to conclude that progesterone in the time and dosage schedule employed lacks any material effect on the clinical course of the disease. Histopathological study of serial biopsy specimens reveals no deviations from the pathological changes usually seen in such tumors. A report to this effect is in preparation for publication.

D) Proposed study: We propose to evaluate the possible effect of more massive dosage of progesterone given by intravenous route in propylene glycol plus human serum albumin. We also propose to study the effect of androgens, estrogens, and corticoids in this relationship. Nor-progesterone will be evaluated when available, as well as the anti-estrogenic compound allopregnane 21-ol-3,20 dione.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

PROJECT III

10. NCI-8020
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$ 18,650	6	0	6	2	0	2	1000
FY 1956	$ 18,550	6	0	6	2	0	2	1000

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Surgery Branch, NCI - Dr. Smith and staff.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT III

15. NC1-802-C
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Letter to Editor "Progesterone and Carcinoma of the Cervix" by Roy Hertz and J. Keith Cromer. J.A.M.A. 154: 1114, 1954 (March 27).

Endocrinological Background of Patients with Endometrial Hyperplasia or Cancer of the Fundus, by Roy Hertz. Presented at American Cancer Society Symposium, New York City, October 18, 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

PROJECT REPORT FORM
PROJECT IV

1. National Cancer Institute (INSTITUTE) 2. Endocrinology Branch (LABORATORY OR BRANCH)

3. Endocrinology Service (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-803C (SERIAL NO)

6. The Nature of Hormone-Producing Tumors of Pituitary, Adrenal, Ovary, Testis, Pancreas, Parathyroid and Chorion (PROJECT TITLE)

7. Roy Hertz and William W. Tullner (PRINCIPAL INVESTIGATOR(S))

8. James Pittman, Leslie DeGroot, Alexander Breslow and Harold Altman (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION A) Objectives: 1. To learn more about the basic metabolic phenomena involved in the process whereby neopasms in hormone-producing organs produce highly excessive amounts of hormone, resulting in such syndromes as Cushing's Disease, Acromegaly, Hyperparathyroidism, Hyperadrenalism, and similar endocrinopathies.

2. To maintain in tissue culture hormone-producing tissues and to ascertain the factors involved in quantitative hormone production in vitro.

B) Methods: Hormone producing tumors from patients with one of the above-named syndromes are obtained at surgery and carried in tissue culture. The supernatant fluid from the cultures are tested for hormonal activity by appropriate bioassay methods and the quantity recovered compared with the content of a piece of tissue exactly equivalent to that originally placed in culture.

Patients presenting such syndromes are completely characterized endocrinologically and metabolically before and after therapy in order to ascertain the relative effects of various forms of therapy.

C) Major findings: 1. Hydatid mole tissue from 9 cases has been maintained in tissue culture for as long as 22 months with continuous production of chorionic gonadotropic hormones. Complete morphological dedifferentiation of certain cultures was nevertheless accompanied by continued hormone production.

2. Nine patients with hydatid mole have been shown to lack the histidinuria seen in normal pregnancy. This suggests a possible differential test for distinguishing between hydatid mole and normal pregnancy - a clinical problem of considerable magnitude.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Attachment 1 (C) (con't.) PROJECT IV

3. A case of sexual precocity in a six year old boy has been shown to be due to an androgen producing tumor of the testicle.

D) Proposed studies: We propose to continue the above studies. We will emphasize a detailed study of the hormonal profile of patients with acromegaly before, during and after radiation therapy in order to elucidate the mechanism whereby x-ray frequently halts the progress of this deforming disease. Special emphasis will be placed upon the determination of growth hormone in the plasma by special assay techniques still being developed. (See Project IX).

Pancreatic islet cell tumors will be given special consideration with a view to developing an insulin-producing strain of cells for practical insulin production.

Extended attempts to carry heterologous hormone-producing tumors in cortisone-treated animals will be undertaken in addition to the tissue culture work.

PROJECT REPORT FORM (Cont'd)

PROJECT IV

10. NC1-803 C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$33,241	6	1	7	3	1	4	1000
FY 1956	$33,143	6	1	7	3	1	4	1000

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Dr. Earle's Tissue Culture Unit collaborates in this program.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT IV

15. NCI-803C
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Gonadotrophic Hormone Secretion from Hydatid Mole Grown in Tissue Culture, by Helen K. Waltz, William W. Tullner, Virginia J. Evans, Roy Hertz and Wilton R. Earle. J. Nat. Cancer Inst. 14: 1173, 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

NONE

PROJECT REPORT FORM

PROJECT V

1. National Cancer Institute (INSTITUTE) 2. Endocrinology Branch (LABORATORY OR BRANCH)

3. Endocrinology Service (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-804C (SERIAL NO)

6. Biotin and the Avidin-Biotin Complex in Relation to Normal and Neoplastic Tissue Growth (PROJECT TITLE)

7. Roy Hertz, Frederick G. Dhyse and William W. Tullner (PRINCIPAL INVESTIGATOR(S))

8. Dr. Lloyd Law (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION A) Objectives: 1. To ascertain the role of biotin, a dietary trace factor, in the growth of normal tissue and in the development of neoplasms in animals and man.

2. To learn the normal metabolism of biotin in man and to produce biotin deficiency in cancer patients administering low biotin diets plus concentrates of avidin prepared from egg-white.

3. To develop methods for the cheap and practical preparation of large amounts of avidin for therapeutic trial in selected cancer cases.

B) Methods: 1.Standard methods of protein fractionation of egg white are employed to prepare potent avidin concentrates which are assayed for growth suppressing effect on a biotin requiring yeast organism.

2. Biotin deficiency is produced in mice by feeding egg white and avidin concentrates and the effect of this deficiency on such standardized tumors as transplantable leukemia is determined in collaboration with Dr. Lloyd Law.

3. Patients are carried on diets of known and constant biotin content and the urinary biotin excretion is followed microbiologically. The effects of egg white feeding and avidin feeding are observed upon the patient's clinical nutritional status and upon the urinary excretion of biotin.

C) Major Findings: 1. An excellent, economical method of preparing potent concentrates of avidin has been developed and a stock pile of this material for clinical and experimental use has been prepared.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Attachment 1 (C continued) Project V

2. Initial values for biotin excretion have been determined in several patients and found to be sufficiently constant to permit study of effects of avidin on same. One patient has been almost completely depleted of biotin reserve by avidin but showed no significant clinical effect from this depletion.

D) Significance to the Program of the Institute: Numerous experimental animal studies show a marked impairement in carcinogenicity of certain dyes in biotin deficient animals. Induced biotin deficiency may parallel induced folic acid deficiency in depressing certain specific tumor growths with at least a narrow margin of safety with respect to the tissues of the host. At least this same principle ought to be tested out in relation to biotin.

E) Proposed Course of Study: These studies are to be continued as outlined above. The animal work will emphasize leukemia and hormone-induced tissue growth of the uterus and prostate. The clinical studies will be pursued in patients with lesions suitable for objective evaluation. Appropriate contract arrangements are to be developed with industrial laboratories for the preparation of the large amounts of avidin necessary for the clinical studies. The methods developed by Mr. Dhyse will probably have to undergo some modification for large-scale application.

PROJECT V

10. NCI-804C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$11,958	1	1/2	1 1/2	1	1/2	1 1/2	600
FY 1956	$14,593	1	1	2	1	1	2	600

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Dr. Lloyd Law, Head, Leukemia Studies Section of the Laboratory of Biology, NCI, participates in mouse leukemia studies.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT V

15. NCI-804C
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

A Practical Laboratory Preparation of Avidin Concentrates for Biological Investigation by Frederick G. Dhyse, Proc. Soc. Exper. Biol. & Med. 85: 515-517, 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

NONE

PROJECT REPORT FORM

PROJECT VI

1. National Cancer Institute
INSTITUTE

2. Endocrinology Branch
LABORATORY OR BRANCH

3. Endocrinology Service
SECTION OR SERVICE

4. .
LOCATION (IF OTHER THAN BETHESDA)

5. NCI-805C
SERIAL NO

6. Effect of Steroid Therapy on Metabolism of B-Complex Factors in Cancer Patients
PROJECT TITLE

7. Roy Hertz, John Adams Schricker and Donald Watkin
PRINCIPAL INVESTIGATOR(S)

8. Grant D. Coffman
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION A) Objectives: 1. To determine the effect of protein anabolic action of steroid therapy in cancer patients upon the metabolism of B-complex factors consumed in the diet.

2. To correlate the foregoing with specific therapeutic effects obtained.

B) Methods Employed: Patients are maintained on a fixed 3 day dietary cycle and their dietary intake determined by a paired-tray procedure. Continuous urine collection provides specimens for determining urinary excretion of nitrogen, biotin, riboflavin, niacin, folocin, and pantothenate (See project I).

C) Proposed Course of Project: It is proposed to develop and extend these studies as a new project largely by continuing studies already in progress. Observations would be made in suitable patients with androgen-induced protein-anabolism and in cortisone-induced protein-catabolic states. In this manner the relationship of B-complex balance to nitrogen balance may be determined with a view to clarify the mechanism of action of these steroids on protein metabolism generally.

As facilities become available the paired tray technique is to be replaced by full metabolic balance study. This more exact technique will provide necessary control data regarding the differences between androgen-induced nitrogen retention and simple retention following increased intake.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

10. NCI-805 C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$23,916	3		4	2	1	3	
FY 1956	$29,186	3	2	5	2	2	4	400

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Dr. Donald Watkin, General Medicine Branch, is collaborating in completing the studies on patients on the complete balance regime.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM
PROJECT VII

1. National Cancer Institute — INSTITUTE
2. Endocrinology Branch — LABORATORY OR BRANCH
3. Endocrinology Service — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-806-C — SERIAL NO

6. Endocrine Characterization of Females with Cancer of the Larynx
PROJECT TITLE

7. Roy Hertz, Morris Graff and Lois F. Hallman
PRINCIPAL INVESTIGATOR(S)

8. James Pittman, Harold Altman, Alexander Breslow and Leslie DeGroot
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION A) Objectives: 1. To determine the endocrinological characteristics of the relatively rare female patient with cancer of the larynx in order to assess the role of endocrine factors in the pathogenesis of this disease.

B) Methods Employed: Women with proven cancer of the larynx will be hospitalized and a complete endocrine evaluation made by: (1) recording a complete pubertal, menstrual, and reproductive history and any other pertinent endocrine facts regarding the patient and her family; (2) complete physical appraisal of endocrine make-up with emphasis on obesity, masculinity, size of clitoris, hirsutism, voice pitch, acne, baldness, vaginal smear and other endocrine factors; (3) determination of urinary corticoids, estrogens, ketosteroids, and gonadotropins; also glucose tolerance, B.M.R., protein bound iodine, eosinophile response test and other measures of endocrine functions.

These data would be collected in order to determine whether there is any evidence of gonadal or adrenal androgenization of the female who gets cancer of the larynx as compared with normal females of the same age group.

C) Major Findings: Preliminary superficial clinical observation of 2 females with cancer of larynx has revealed no striking difference.

D) Significance to the Program: Literature review indicates that the sex ratio for cancer of the larynx is variously estimated as between 16 to 1 and 200 to 1. In any case it is clear that females are relatively rare among patients with laryngeal cancer.

The larynx is a secondary sex organ in that its pubertal differentiation is hormonally dependent. Moreover, androgen administration readily alters the larynx in the human female but no case of cancer of the larynx in the human female has yet been reported following androgen administration.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT VII

10. NC1-806C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	0	0	0	0	0	0	0	0
FY 1956	$ 33,143	7	1	8	3	1	4	300

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

none

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No entries for 14, 15, 16, and 17

PROJECT REPORT FORM
PROJECT VIII

1. National Cancer Institute (INSTITUTE) 2. Endocrinology Branch (LABORATORY OR BRANCH)

3. Endocrinology Service (SECTION OR SERVICE) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-807c (SERIAL NO)

6. Methods of Steroid Analysis in Urine and Blood from Cancer Patients
PROJECT TITLE

7. Morris Graff
PRINCIPAL INVESTIGATOR(S)

8. Silas Jackson, Foster Burnett and Maurice Moore
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION A) Objectives: To develop and improve the chemical methodology for determination of urinary corticoids and ketosteroids and blood corticoids, estrogens, and androgens.

B) Methods Employed: Classical methods of reagent and enzymatic hydrolysis, chromatographic adsorption and elution, paper chromatography, and spectrophotometric determination of specifically developed chromogens. Chemical methods to be correlated with equivalent bioassays available under Project IX.)

C) Major Findings: Methods for urinary ketosteroids and corticoids have been developed and perfected and are now adaptable for clinical studies.

Blood corticoid methods have been investigated and an improved method devised which is now available for clinical research studies involving assessment of adrenal corticoid function.

D) Significance to the Program: These methods are basic to the endocrinological study of patients with cancer of the breast, prostate, cervix, and larynx.

E) Proposed Course: The further extension of methodological investigation is proposed especially for blood estrogen and urinary estrogen by fluorometric methods. Extended investigation of the use of the Anthrone reagent in steroid analysis is planned.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

PROJECT VIII

10. NCI-8072
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$28,578	3	1	4	2 1/2	1	3 1/2	0
FY 1956	$28,506	3	1	4	2 1/2	1	3 1/2	0

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

PROJECT VIII

15. NC-807c
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

None

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

Mr. Graff is appointed lecturer at Georgetown Medical School and will give a course of lectures on steroids.

PROJECT REPORT FORM

PROJECT IX

1. National Cancer Institute (INSTITUTE) 2. Endocrinology Branch (LABORATORY OR BRANCH)

3. Endocrinology Service (SECTION OR SERVICE) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NCI-808 (SERIAL NO)

6. Endocrine Factors Governing Hormone-Induced Tissue Growth and Tumor Formation in Animals; Bioassay of Blood and Urine from Cancer Patients (PROJECT TITLE)

7. William Tullner, Donald Spencer, Silvio Bruzzone, Lois Hallman (PRINCIPAL INVESTIGATOR(S))

8. F. G. Dhyse, L. DeGroot, A. Breslow, Harold Altman, James Pittman (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION A) Objectives: 1. To gain basic information concerning endocrinological and nutritional factors governing hormonally conditioned growth and involution in organs such as the uterus, breast and prostate which are the frequent seat of hormone-sensitive neoplasms.

2. To develop and improve bioassay methods for the quantitation of hormones in the blood and urine of patients with cancer who are either receiving various forms of hormonal therapy or who present unusual hormonal patterns.

3. To determine the biological properties of steroid and related compounds in terms of their potential usefulness in cancer therapy.

B) Methods Employed: Standard methods of biological study are employed. These include: (1) the maintenance of animals of various species on specified hormonal or nutritional regimens; (2) daily observations of physical and behavioral changes in such animals; (3) complete autopsy of test animals with detailed weighing and chemical analysis and histological study of specific organs; (4) surgical alteration of the endocrine status of animals by gonadectomy, adrenalectomy, hypophysectomy, thyroidectomy, parabiosis, hysterectomy and other operative procedures.

C) Major Findings: These are detailed in the publications listed under Item 16. They may be briefly enumerated as follows:

1. Histochemical studies by Dr. Bruzzone show that a rapid accumulation of DNA in the nucleolus of the epithelial cells of the rat endometrium is one of the first effects of estrogenic stimulation. In addition, the nucleolus shows duplication under estrogen stimulation. These phenomena reflect a very early and sensitive response to estrogen. The chronological factors and the quantitative aspects of these changes remain to be worked out.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Attachment 1 (C continued) Project IX

2. Additional studies on the inhibition of estrogen-induced tissue growth by folic antagonists show that the uterus of the rat may be rendered resistant to this inhibition by prior sensitization with estrogen alone. It would appear that some of the uterine cells acquire the capacity to overcome the anti-folic inhibition such as do the white cells of the leukemic patient or animal. Various nutritional studies are to be undertaken to attempt to restore sensitivity to the resistant uteri. We regard this as of fundamental importance since it shows the capacity of a tissue to adapt to an alternative metabolic system in order to effect a hormonal response.

3. The biological properties of Amphenone in relation to adrenal cortical function have been extensively studied. Those findings were presented before the Laurentian Hormone Conference. Conclusive evidence of interference with corticoid synthesis by Amphenone in the dog was presented. These data are derived from quantitative corticoid determination in the adrenal-vein blood of the hypophysectomized dog given variable doses of ACTH both with and without Amphenone. A marked suppression in response of the adrenal to exogenous corticoid is readily demonstrated.

A series of analogues of Amphenone have been synthesized and studied. These include alpha, beta, dimethyl-4 4'stilbenediamine, the dimethylamino derivative of Amphenone, the oxine derivative of Amphenone, and the butanone derivative of Amphenone. These compounds share the biological properties of Amphenone and further differential studies are being made with respect to relative toxicity and central nervous system depressant effect. Twenty-five related compounds have been found to be inert, indicating a relatively high degree of specificity for these interesting endocrinological effects.

Extended work with these and related substances is projected with a view to deriving a non-toxic, specific inhibitor of adrenal-cortical function.

Chromatographic analysis of the steroid content of the adrenal vein blood of the Amphenone-treated animal is also to be carried out.

4. Further studies on potent anti-gonadotrophic sera have led to some degree of purification of the active inhibitory material. These efforts are to be extended by means of specific immunochemical adsorption and elution techniques now being developed.

5. Additional steroids have been screened for possible dissociation of pituitary depressant action from other biological effects. The studies on the biological properties of allopregnane, 21-ol-3,20 dione and Reichstein's compound S have been completed and reported. Assays of the new corticoids, metacortandrosin and metacortandrolone have indicated no material dissociation of pituitary depressant action and enhanced corticoid action.

6. Extension of studies on the local catabolic function involved in the involution of hormone sensitive organs following gonadectomy substantiate the complete quantitative and chronological independence of this process from general catabolic effects on the body as a whole.

Substantial progress has been made on a sensitive and highly specific assay for luteinizing hormone. A normally cycling female rat is followed by vaginal smears

until she is found to be in the pre-ovulatory or pre-estrus phase which is characterized by the appearance of very large numbers of large rounded, nucleated epithelial cells in the vaginal smears. Our previous studies had shown that hypophysectomy at this point will abort the succeeding ovulation. Since this ovulation depends entirely on luteinizing hormone, a sensitive and specific assay is based on the restoration of ovulation by injection of such hormone preparations. It is hoped that this will permit us to assay for this hormone in the blood and urine of cancer patients under steroid regimens.

A strain of guinea pigs has been identified which has a large number of hypogonadal males. These animals have normal mating behavior but have complete aspermia and extremely atrophic testes. A complete endocrine, behavioral, and anatomical study of these animals is being undertaken in collaboration with Dr. W. C. Young, Prof. of Anatomy at the University of Kansas.

Proposed Course: It is expected to continue and extend the foregoing studies along the lines already indicated.

PROJECT REPORT FORM (Cont'd)

PROJECT IX

10. NCI-808
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$42,566	8	1	9	4	1	5	0
FY 1956	$43,418	8	1	9	4	1	5	0

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

4. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT IX

15. NCI-108
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

1. A Comparison of the Biological Action of Allopregnane-21 ol,3,20 dione and Progesterone by Tullner, W. W., Hertz, R. and Raffelt, E. Endocrinology 55: 762, 1954.

2. Biolgical Demonstration of Estrogenic Substances Excreted in Human Saliva, by Stanley Green, Proc. Soc. Exper. Biol. & Med. 86: 53, 1954.

3. Studies on Amphenone and Related Compounds, by R. Hertz, W. Tullner, J. A. Schricker, F. G. Dhyse and L. Hallman. Presented at the Laurentian Hormone Conference, Mont Tremblant, Canada, September 1954.

4. Effect of Amphenone B on Liver Glycogen of the Rat, by Lois Hallman (in preparation).

5. Quantitative Aspects of Regression of Prostate and Uterus of the Rat Following Hormonal Deprivation, by Roy Hertz. Presented at the Third Pan American Congress of Endocrinologists, Santiago, Chile, Novembef 27, 1954.

6. The Inhibition of Estrogen-Induced Growth in the Chick Oviduct by Reichstein's Compound "S" by Roy Hertz and Wm. W. Tullner (In press).

7. Lack of Effect of Cortisone on the Inhibition of Endogenous Gonadotropin by Anti-gonadotrophic sera, by Roy Hertz and Wm. W. Tullner (In press).

8. Anti-Metabolite Principle as Applied to the Hormonal Control of Neoplasms, by Roy Hertz. Monograph on "Anti-Metabolites and Cancer," published by A.A.A.S.

9. The Pharmacological and Biological Effects of Amphenone, by Roy Hertz. Lecture presented before Endocrine Section of the Argentine Medical Society in Buenos Aires, November 29, 1954.

(continued on attachment 3)

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

Dr. R. Hertz: invited to serve as U.S. delegate to the Third Pan American Congress of Endocrinologiests, Santiago, Chile, November 21, 1954.

Dr. Hertz and colleagues invited to present review paper at Laurentian Hormone Conference, September 1954.

Dr. R. Hertz: Intited to lecture before the Argentine Medical Society and given honorary membership in this organization, Buenos Aires, November 29, 1954.

" " " Presented lecture to Medical School, University of Brazil, Rio de Janeiro, December 2, 1954.

" " " Presented Abraham Hymanson Memorial Lecture at Beth Israel Hospital, New York, December 20, 1954.

Dr. Hertz and colleagues invited to participate in the work of Subcommittee of A.M.A. "Comparison of the Biological Properties of Steroids and Hormones." (con't.)

PROJECT REPORT FORM

PROJECT X

1. National Cancer Institute
INSTITUTE

2. Endocrinology Branch
LABORATORY OR BRANCH

3. Endocrinology Service
SECTION OR SERVICE

4.
LOCATION (IF OTHER THAN BETHESDA)

5. NCI-809
SERIAL NO

6. The Preparation of Crude Natural Extracts of Hormonal Activity from Ovary, Testis, Adrenal and Pituitary and their Biological and Clinical Characterization.
PROJECT TITLE

7. To be recruited.
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION Objectives: To prepare for biological and clinical assay crude preparations of hormonally active tissues in order to determine their effect upon tumors in hormone-sensitive organs. We know that the crystalline steroids now so widely used represent only partial elements of the over-all activity of the various endocrine organs and that even optimum mixtures of them do not reproduce the characteristic effect of the total internal secretion of the endocrine gland itself. Such effects may be more profound and more desirable than those obtained by the separate synthetic crystalline factors.

Methods: 1. Standard chemical methods of total lipid and protein extraction of freshly prepared tissues to be collected at the slaughter house and processed immediately

2. The application of bioassay techniques to such preparations to determine their endocrine effectiveness and their toxicity.

3. Clinical trial of non-toxic, active materials in suitable patients with endocrine deficiency or cancer of breast, prostate or cervix.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

PROJECT X

10. NCJ-809
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	0	0	0	0	0	0	0	0
FY 1956	$ 19,911	1	2	3	1	2	3	0

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

none

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

no entries for 14, 15, 16, and 17

PROJECT REPORT FORM

PROJECT X

1. National Cancer Institute
INSTITUTE

2. Endocrinology Branch
LABORATORY OR BRANCH

3. Endocrinology Service
SECTION OR SERVICE

4.
LOCATION (IF OTHER THAN BETHESDA)

5. NCI-809
SERIAL NO

6. The Preparation of Crude Natural Extracts of Hormonal Activity from Ovary, Testis, Adrenal and Pituitary and their Biological and Clinical Characterization.
PROJECT TITLE

7. To be recruited.
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION Objectives: To prepare for biological and clinical assay crude preparations of hormonally active tissues in order to determine their effect upon tumors in hormone-sensitive organs. We know that the crystalline steroids now so widely used represent only partial elements of the over-all activity of the various endocrine organs and that even optimum mixtures of them do not reproduce the characteristic effect of the total internal secretion of the endocrine gland itself. Such effects may be more profound and more desirable than those obtained by the separate synthetic crystalline factors.

Methods: 1. Standard chemical methods of total lipid and protein extraction of freshly prepared tissues to be collected at the slaughter house and processed immediately

2. The application of bioassay techniques to such preparations to determine their endocrine effectiveness and their toxicity.

3. Clinical trial of non-toxic, active materials in suitable patients with endocrine deficiency or cancer of breast, prostate or cervix.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

PROJECT REPORT FORM (Cont'd)

PROJECT X

10. NCJ-809
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	0	0	0	0	0	0	0	0
FY 1956	$ 19,911	1	2	3	1	2	3	0

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

none

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

no entries for 14, 15, 16, and 17

PROJECT REPORT FORM

PROJECT XI

1. National Cancer Institute — INSTITUTE
2. Endocrinology Branch — LABORATORY OR BRANCH
3. Endocrinology Service — SECTION OR SERVICE
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-810C — SERIAL NO

6. Studies in Thyroid Function — PROJECT TITLE

7. Dr. Max Sherer — PRINCIPAL INVESTIGATOR(S)

8. Harold Altman, Betty Siefring, Paul Marks — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION A) The Hepatic Clearance of Plasma Protein Bound Iodine in Man: The objective of this project is to determine, in man, the role of the liver in the excretion, conjugation and utilization of thyroid hormone. Results obtained are to be compared with experimental information available from similar work done in animals (rat, mouse, cat, dog).

Methods Employed: Hepatic vein catheterization in man; serum or plasma protein bound iodine determinations, hepatic blood flow measurements employing the bromsulfaphthalein technique.

Major Findings: The major findings of this project during the past year have been the demonstration of a significantly lower plasma protein bound iodine concentration in blood from the hepatic veins of humans as compared to the concentration of plasma protein bound iodine in the peripheral veins or femoral artery blood of the same subject. This enables one to calculate the amount of protein bound iodine removed by the liver and gives a measure of the entero-hepatic circulation of thyroxine; the first such information available in man.

Proposed Course: It is proposed for the next calendar year to continue studying the fate of thyroxine in the liver of man by utilizing radioiodine tagged thyroxine. This compound and its metabolic products will be traced into the liver, bile, gastrointestinal tract and hepatic vein of man. Metabolic degradation and conjugation products of labelled thyroxine will be studied by means of paper partition chromatography. The measurements of hepatic plasma protein bound iodine clearance will be extended.

B) The Effects of Various Steroids on Thyroid Function and the Metabolic Fate of Thyroxine: The effedt of estrogens, androgens and corticoids on thyroid function and the metabolic fate of thyroxine.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Attachment 1. Item 9 continued PROJECT XI

Methods Employed: Radioiodine 24 hour tracer uptakes; thyroid gland hormone secretion rates; paper chromatography of protein bound radioiodine compounds, serum protein bound iodine determinations; disappearance rate measurements of blood radiothyroxine; serum protein thyroxine binding capacities.

Major Findings: a) Estrogens cause a significant rise in serum protein bound iodine without a concomitant rise in 24 hr. tracer radioiodine uptake of the thyroid gland; b) secretion rate: in two patients, so far, one has had no change in secretion rate with estrogen administration; one has had slowing of secretion rate with estrogen administration; c) testosterone causes a marked fall in serum protein bound iodine and 24 hr. radioiodine tracer uptake by the thyroid gland; d) Metacortandrocin and metacortandrolone, corticoids similar to hydrocortisone, cause a marked fall in uptake and serum protein bound iodine; e) paper partition chromatography has failed to reveal any qualitative change in the iodinated compounds released by the thyroid under the influence of the steroids mentioned above.

Proposed Course: It is proposed, during the next calendar year to extend these studies to a greater number of cases and to employ radioactive iodine labelled thyroxine for tissue utilization studies during control and steroid administration periods in the same patient.

C) Extra Thyroidal effects of Thyroid Stimulating Hormone: TSH Effect on Thyroxine to Triiodo-thyronine Conversion: Methods employed: Measurements of serum or plasma protein bound iodine, rate of radio thyroxine utilization in vivo by scintillation gamma counting of blood protein fractions in sequential fashion.

Major findings: One case studied so far over 2 week period. No change in thyroxine utilization rate was found.

Proposed Course: Extension of present study to several patients including radio-thyroxine utilization studies.

D) In Vitro Conversion of Thyroxine to Triiodo-Thyronine by Surviving Tissue Slices and Homogenates, and the Factors Influencing this Interconversion: Objectives: To confirm and extend the work of Albright and Larsen who demonstrated in vitro conversion of thyroxine to triiodo-thyronine by rat kidney slices. To isolate the enzyme systems responsible for the deiodination involved and to ascertain the factors influencing this enzyme's action.

Methods Employed: Surviving tissue slices, tissue cultures and homogenates incubated with radioiodine labelled l-thyroxine. The products of these incubations are then analyzed by paper partition chromatography.

Major Findings: No conversion to triiodo-thyronine by mouse liver tissue cultures; slight appearance of triiodo thyronine after incubation with monkey surviving kidney slices and homogenates in phosphate buffer.

Proposed Course: Continued work with monkey and rat tissue similar to that noted above.

PROJECT REPORT FORM (Cont'd)

PROJECT XI

10. NCI-810 C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$ 14,591	3	1	4	1	1	2	300
FY 1956	$ 17,252	3	2	5	1	1 1/2	2 1/2	300

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Dr. Paul Marks, NIAMD is cooperating in this project.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

NCI-810C

Attachment 2. Item 9 continued PROJECT XI

Significance of the program to the Institute: Further elucidation of our knowledge of the mechanism of action of the steroid hormones on peripheral tissues may be expected to help explain some of the favorable and unfavorable effects on cancer of the breast, prostate and uterus.

PROJECT REPORT FORM (Cont'd)

PROJECT XI

15. NC-810 C
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

1. Invited to give series of lectures at Providence Hospital, Washington, D. C. on endocrinological problems.
2. Elected member of A.A.A.S.
3. Guest lecturer, Alexandria Hospital, Alexandria, Va. "Latest Advances in Thyroid Physiology."

PROJECT REPORT FORM

PROJECT XII

1. National Cancer Institute — INSTITUTE
2. Endocrinology Branch — LABORATORY OR BRANCH
3. Endocrinology Service — SECTION OR SERVICE
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-811C — SERIAL NO

6. Studies on thyroid adenomata and related problems in pituitary-thyroide hypothalamic relationships. — PROJECT TITLE

7. Monte A. Greer — PRINCIPAL INVESTIGATOR(S)

8. Leslie J. DeGroot, Eleanor Siperstein, Howard Erwin, Robert O. Scow — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION A) Correlation of histologic morphology and metabolism of simple goiter with its response to thyroid therapy:

Objectives: To determine whether these is any correlation between the microscopic appearance and metabolic activity of various types of simple goiter with their diminution in size when desiccated thyroid therapy is administered.

Methods employed: Studies of the metabolic activity of simple goiter are made with I-131 tracer studies. A wedge biopsy is then made of the gland and 3 weeks later administration of 3 grains daily of desiccated thyroid begun. At the end of a 4-6 month period a final biopsy is performed. In the case of single nodules, the endire nodule is removed at the end of the therapy period.

Major findings: During the past year, only 4 such patients have been studied. These included cases of simple goiter, chronic thyroiditis, and single thyroid adenomas. All goiters thus far studied have experienced considerable diminution in size.

Proposed course: A continuation of the study as outlined above is planned for the coming year.

B) Isolation and identification of precursor of naturally occurring antithyroid compound, 1-5 vinylthiooxazolidone.

Objectives: To isolate, crystallize, and identify the structure of the precursor of the only known naturally occurring antithyroid substance, vinylthiooxazolidone. The precursor may be of a type that is more widespread among various plant foods than is now believed.

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

Attachment 1. Item 9 (B continued). PROJECT XII

Methods employed: Brassica seed extracts are fractionated by various procedures, including column chromatography, after first heat denaturing the thioglycosidase which specifically hydrolyzes the precursor. Assay of the fractions is greatly facilitated by following the UV abortion spectrum. The precursor has a specific peak absorption at 2270 A°. Crystallization and identification of the pure compound are by standard chemical and microchemical procedures.

Major findings: During the past year, the precursor has been purified and crystallized. It has been found to be a thioglycoside having a molecular weight of about 1000, m.p. 130° C. Both sodium, potassium, and SO_4— have been identified in the molecule. The glycone has been tentatively identified as a fructose polymer.

Proposed course: It is planned to continue with degradation experiments to work out the structure of the compound and to determine by animals and human assay whether any biologic system not containing the specific thioglycosidase fround in Brassica members is capable of liberating thiooxazolidone from it.

C) Stimulation of the central nervous system of rats by radiofrequency transmission.

Objectives: To devise apparatus capable of stimulating the central nervous system, particularly the hypothalamus, of rats. The animals are to be completely unrestrained and without any external connections to the pulse generating equipment. It is hoped to produce hyperfunction of the pituitary by stimulation of those areas which, when destroyed by electrolytic lesions, result in decreased hypophysial function. In this way, considerable information may be gained of neuro-endocrine interrelationships.

Methods employed: A technique has been devised for fixing radio receivers to the skull of rats, with stimulating electrodes buried according to calculation in various parts of the hypothalamus. A ratio sending and receiving apparatus has been devised to carry variable pulses to the hypothalamic electrodes. The receiver in the rat is entirely subcutaneous. It is possible to stimulate 36 or more rats at one time and with variable intensities and frequencies.

Changes in hypophysial function are measured by gross and cytologic changes in the pituitary and target endocrine glands and by noting alterations in the functional activity of the target glands.

Concomitantly observations are made of the behavioral changes produced in the animals by stimulation.

Major findings: During the past year, the major emphasis has been on developing equipment and techniques to a satisfactory level. It has been discovered that stimulation of the hypothalamic area lateral and just posterior to the paraventricular nuclei will induce marked drinking behavior in the rats. Stimulation slightly posterior to this area will induce marked eating behavior.

Proposed course: The major emphasis during the coming year will be devoted to working out the "bugs" in the stimulation system and develop it to the point where stimulation can be continued for several weeks without any appreciable loss of response in the stimulated animal.

Attachment 2. Item 9 (continued) PROJECT XII

D) Study of the structure and function of heterotopic compared with normal pituitaries.

Objectives: To determine the alterations in structure and function produced in the pituitary gland by transplanting it to an abnormal site and to determine what factors are involved in the changes thus produced.

Methods employed: Pituitaries from new born mice are transplanted into the eye or kidney of genetically homogeneous mature mice. The hosts are hypophysectomized one week later. Three weeks following hypophysectomy the function of the heterotopic pituitaries is measured by following changes in body growth and changes in the metabolism and structure of the target endocrine glands. Pituitary cytology is studied by the various advanced techniques now available.

Major findings: 1) Heterotopic pituitaries are able to maintain thyroidal I-131 metabolism at a level approximately equal to that in intact animals. They are not able to maintain thyroid weight or the weight of other target endocrine organs significantly above that of hypophysectomized animals. Raising or lowering the level of circulating thyroxin will cause marked changes in the secretion of the pituitary hormone controlling thyroidal I-131 metabolism in these animals without having any significant effect on thyroid weight.

2) Glycogen has been demonstrated in the pituitary for the first time. This is present particularly in the younger glands and in highest concentration in the pars tuberalis.

3) The differences in cytology of heterotopic compared to in situ pituitaries has been compared for various ages. One of the most striking differences is the marked diminution in chromophiles in the heterotopic glands. There is also a marked hypertrophy of the pars intermedia comparable to that seen following hypothalamic lesions

Proposed course: The major effort during the coming year will be devoted to a study of whether function of the heterotopic pituitaries can be increased by transplanting various parts of the central nervous system, particularly the hypothalamus, in direct connection with the pituitary transplants. A system has already been devised for making transplants of considerable size to the kidney of adult mice.

It will also be determined whether the heterotopic pituitaries secrete the substance necessary for ovarian and uterine response to chorionic gonadotropin. A further study will involve whether they can concentrate isotopically labelled triiodothyronine to as marked an extent as do normal glands.

E) The effect of hypothalamic lesions on pituitary function.

Objectives: To determine what effect electrolytic lesions in various parts of the hypothalamus have upon the regulation and secretion of the various hormones produced by the pituitary gland.

Methods employed: Electrolytic lesions are made in the hypothalamus of the rats by means of a specially designed stereotaxic instrument. Following a period of 1-3 weeks

Attachment 3. Item 9 -(E continued) PROJECT XII

for recovery, studies are made of the ability of the pituitary to secrete the various trophic hormones. These determinations are made by studying the alterations of structure and function produced in the target endocrine glands by various stimuli. For example, thyrotrophin secretion is studied by the change produced in thyroid size and I-131 metabolism by chronic administration of propylthiouracil.

Major findings: During the past year it has been found that the location of the hypothalamic area controlling thyrotrophin secretion in the rat lies in the midline between the paraventricular nuclei and the median eminence. Destruction of all or part of this area will markedly interfere with thyrotrophin secretion.

It has also been found that although lesions in the above area will prevent the goitrogenic response to amphenone administration, they will not prevent the usual concomitant adrenal hypertrophy. This provides further evidence that the hypothalamic areas controlling the various pituitary functions are discrete.

Proposed course: It is planned to make a study of the location of the hypothalamic area responsible for maintaining normal growth of the pars intermedia of the hypophysis. Material from over 500 rats with hypothalamic lesions has already been assembled and the remaining requirement is to determine how far from the hypophysial stalk it is necessary to place lesions in order to avoid the gross and bizarre hypertrophy of the pars intermedia which occurs with lesions close to the stalk.

It is also planned to investigate the area controlling ACTH secretion more thoroughly and to see if it is possible to place lesions that will interfere with ACTH secretion as much as previous lesions have been interfered with thyrotropin secretion.

F) Study of the mechanism of action of stable iodine in thyrotoxicosis.

Objectives: To determine the mechanism of action of stable iodine in ameliorating thyrotoxicosis and, secondarily, to gain some insight into the pathogenesis of thyrotoxicosis.

Methods employed: Thyrotoxic and euthyroid subjects are given a 100 microcurie tracer dose of I-131. Twenty-four hours later the administration of 30-60 mg. tapazole every 8 hours is begun to prevent reaccumulation in the thyroid of I-131 broken down from endogenously labelled thyroxin. In vivo counting over the thyroid is made by means of an externally placed scintillation counter. The thyroidal secretion rate can thus be determined by plotting on semilog paper.

When the rate has been established, 300 mg. per day of NaI are added to the regimen. When the maximum effect from iodine has been obtained, 10-100 units of thyrotrophin are given daily for a period of several days.

In the case of the control euthyroid subjects, thyrotrophin was frequently given before iodine administration was begun.

Attachment 4. Item 9 (F continued) PROJECT XII

Major findings: It has been found that iodine interferes with the release of hormone from the thyroid gland in thyrotoxic subjects but has little, if any, effect in euthyroid subjects. It has also been found that iodine does not interfere with the stimulation of euthyroid glands by exogenous thyrotrophin.

These results suggest that iodine acts in hyperthyroidism by inhibiting the release of thyrotrophin from the pituitary. Therefore, hyperthyroidism must be a suprathyroidal disturbance and not solely a disease of the thyroid gland.

Proposed course: The project is at present almost completed.

Significance to the program of the Institute: Studies of thyroid adenomata will have a direct bearing on the problem of the pathogenesis of thyroid cancer. In addition, further knowledge of the pituitary-thyroid interralationship will provide potential means of controlling abnormal thyroid growth.

PROJECT XII

10. NCI-811-C
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$23,916	3	1	4	2	1	3	600
FY 1956	$23,868	3	1	4	2	1	3	600

12. BUDGET ACTIVITY:

RESEARCH [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Dr. Robert O. Scow, NIAMD, collaborates.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

PROJECT REPORT FORM (Cont'd)

PROJECT XII

15. NCI-811C
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

Scow, R. O. and M.A. Greer. Effect on the thyroid gland of experimental alteration of the level of circulating thyroxin in mice with heterotopic pituitaries. Endocrinology (In press).

Method for increasing the accuracy of the radioiodine uptake as a test for thyroid function by the use of desiccated thyroid, by M. A. Greer and G. Edward Smith. J. Clin. Endocrinol. & Metab. 14: 1374, 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

Greer, M.A.: Invited to participate in meeting of Laurentian Hormone Conference, Mt. Tremblant, Canada, Sept. 1954.

Invited to give seminar at Howard University, November, 1954. (Dr. M.A.Greer)

M. A. Greer, To lecture - post-graduate course at George Washington University Hospital, October 1954.

M. A. Greer: Invited to address Thyroid Study Club at Atlantic City, May 2, 1954.

M. A. Greer: Invited to prepare a section on Chemical Composition of Thyroid in the Handbook of Biological Statistics by the Nat. Res. Council.

PROJECT REPORT FORM

1. National Cancer Institute (INSTITUTE)
2. Pathologic Anatomy Branch (LABORATORY OR BRANCH)
3. Surgical Pathology and Postmortem Service (SECTION OR SERVICE)
4. Bethesda, Maryland (LOCATION (IF OTHER THAN BETHESDA))
5. NCI-850 (SERIAL NO)

6. Pathologic Anatomy in the Clinical Center, National Institutes of Health (PROJECT TITLE)

7. Dr. Harold L. Stewart, Chief, Pathologic Anatomy Branch
Dr. Louis B. Thomas, Head, Surgical Pathology and Postmortem Service (PRINCIPAL INVESTIGATOR(S))

8. Drs. Ellsworth Alvord, William Banfield, Raymond Christensen, Charles Congdon, (OTHER INVESTIGATORS)
John Edgcomb, Benjamin Highman, Albert Hilberg, Ed Lerner, Ladd Loomis, Leon Sokoloff, Harold Stanley, Louis Thomas

9. PROJECT DESCRIPTION

Objectives: The objectives of the Surgical Pathology and Postmortem Service in the Clinical Center are twofold: first, to furnish a diagnostic service in autopsy and surgically removed tissues, and second, to aid from a morphological standpoint the various clinical research problems which are under study in the Clinical Center. To accomplish this, there are in addition to the professional staff, numerous other investigators listed under item 8 who from time to time work on particular cases or particular problems in pathologic anatomy in the Department.

Methods employed: The methods used are those standard methods which involve description of organs and tissues, fixing and sectioning of this material and the preparation of histological slides. These methods include preparation methods usually used in a Pathologic Anatomy Department and in addition because of the many problems of investigators, the use of a wide variety of special staining procedures.

Patient Material: The appended chart shows in detail the human material in the laboratory during 1954. It lists the 76 autopsies performed with an Institute breakdown, and shows that during the year over 8000 slides were cut. Nearly 5000 of these were stained with a variety of stains, chiefly hematoxylin and eosin. One thousand one hundred specimens were accessioned in the Surgical Pathology Service and the Institute breakdown and number of sections prepared can be seen from the chart. Of note is the fact that 66 frozen section reports were made during the time of surgical operation and these were done in the module set aside for that purpose, room 10N218. In addition this laboratory area module was also frequently used to handle tissue under sterile conditions and dissect immediately after surgical removal so that portions of it can be

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

used for special chemical or bacteriological reasons.

Aside from the attempt to provide excellent diagnostic service as far as surgical and postmortem specimens are concerned, there also developed during the year the particularly important function of furnishing certain tissues to scientists in various laboratories at the National Institutes of Health. There are at present approximately 25 scientists at N. I. H. who have current requests for portions of particular tissues or organs on file with the Department and on many occasions it is possible to furnish these investigators with fresh human material for their research. In fact, an average of approximately 12 of these investigators are contacted at the time of each autopsy done in the Department.

The staff of the Pathologic Anatomy Department participates in numerous conferences in the Clinical Center, in order to discuss with clinical colleagues the diagnoses and subsequent course of various patients. To meet this responsibility a total of four weekly conferences have been established in the Department. One of these is the Brain Conference conducted by Dr. Alvord on Monday, an Autopsy Conference on Tuesday, a Joint Pathology Staff Conference on Wednesday and a Surgical Pathology Conference on Friday.

Much of the time and effort of the staff during 1954 was used for moving into the Clinical Center and establishing a smoothly functioning Department. During the coming year the installation and routine operation of the Department should be completed and more effort on the part of the staff can be made for the work-up and study of material examined in the Department.

10. NC1-850
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
		PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	
FY 1955	$30,937	14	5	19	2	2 1/3	4 1/3	0
FY 1956	$30,959	16	5	21	2	2 1/3	4 1/3	0

12. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

No entries for 15, 16, and 17

ANNUAL REPORT OF SURGICAL PATHOLOGY AND POSTMORTEM SERVICE

REPORT OF PATHOLOGIC ANATOMY DEPARTMENT January 1954 to December 1954

INSTITUTE AND TYPE OF PATIENT

Service and Type of Activity	NCI		NHI		NIMH		NIAMD		NMI		NINDB		NIDR		TOTAL		REF.	
	In	FU	In	FU	In	FU	In	FU	In	FU	In	FU	In	FU	In	FU	In	FU
Postmortem-Pathologic Anatomy Service																		
No. of autopsies performed	51		14				2		5		4				76			
Blocks of tissue	2340		715				53		293		118				3519			
Slides	5444		1770				388		630		257				8489			
Slides actually stained	2891		1021				154		371		187				4624			
Surgical Pathology																		
Tissue Specimens	669	17	74	2	4		65		92	1	28	2	42	18	974	126		86
Slides H-E staining	2342	54	229	2	4		292		314	6	72	1	77	41	3330	324		220
Frozen sections	53		2				2		5		4				66			
Special Procedures	1196	9	171				270		360		19		11	5	2027	25		11
Cytodiagnosis Laboratory																		
Vaginal and cervical specimens																		
Accessions																		
Smears																		
Prostatic specimens																		
Accessions																		
Smears																		
Gastric specimens																		
Accessions																		
Smears																		
Bronchial Washings																		
Accessions																		
Smears																		
Misc. Fluids																		
Accessions																		
Smears																		

PROJECT REPORT FORM

1. National Cancer Institute — INSTITUTE
2. Pathologic Anatomy Branch — LABORATORY OR BRANCH
3. Cytodiagnostic Service — SECTION OR SERVICE
4. Bethesda, Maryland. — LOCATION (IF OTHER THAN BETHESDA)
5. NCI-851 — SERIAL NO

6. "Exfoliative Cytology as Applied to Human Diagnostic Problems and as a Research Tool" — PROJECT TITLE

7. Dr. Albert W. Hilberg — PRINCIPAL INVESTIGATOR(S)

8. None — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project title: "Exfoliative Cytology as Applied to Human Diagnostic Problems and as a Research Tool".

Objectives: The Cytodiagnosis Service has a primary objective - the provision of a diagnostic tool to the clinician for the early diagnosis of malignancy or the detection of unsuspected malignancy. In addition attempts are being made to further define and evaluate the cytological criteria of malignancy, the specific differences in components ofmalignant cells, the significance of malignant cells in washings from surgical wounds, and the significance of cytologic changes during endocrine therapy or chemotherapy.

Methods employed: The methods are those of routine Papanicolaou staining and careful cytological screening procedures. These are done with concurrent clinical evaluation. Additional methods employ special techniques of preparation of cytologic specimens for better preservation of cellular components; special staining procedures for specific cellular substances and structures. For example, a fluid aspirated from the pleural cavity is first stained by routine Papanicolaou stains and hematoxylin-eosin stains. In addition this fluid is separated into various fractions by centrigugation, some portions are prepared by clearing with a sodium chloride solution, some portions are suspended in human serum, etc. Specific stains are employed for specific cellular detail such as the Periodic acid Schiff reaction; Best's Carmine stain; Gomori's acid and alkaline phosphatase method; Regaud's method for mitochondria; Laidlaw's Method for Dopa Oxidase; Foot's modification of Bielchowsky's method for reticulum, etc. Comparison studies are made between different smears of exfoliated cells and between smears of exfoliated cells and tissue sections, particularly in instances of therapy problems.

Patient Material (1954 Calendar Year)*

(PLEASE ADD SHEETS AS NECESSARY FOR CONTINUATION)

9. PROJECT DESCRIPTION (Cont.)

and nuclear details as demonstrated in malignant and non-malignant cells from various body sites.

An attempt will be made to provide adequate material and instruction to those physicians of the National Institutes of Health who desire and request such material and instruction in order to further their professional knowledge and skills.

10. NCI-851
SERIAL NO.

11. BUDGET DATA:

	ESTIMATED EXPENDITURES	BUDGETED POSITIONS PROF	OTHER	TOTAL	MAN YEARS PROF	OTHER	TOTAL	PATIENT DAYS
FY 1955	$ 30,389	1	3-4	4-5	1	[illegible]4	[illegible]5	
FY 1956	$ 40,503	1½	4-5	5½-6½	1½	[illegible]5	[illegible]6½	

12. BUDGET ACTIVITY:

RESEARCH ☒ ADMINISTRATION ☐
REVIEW & APPROVAL ☐ TECHNICAL ASSISTANCE ☐

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Surgery Branch, National Cancer Institute
(1 Technician, GS-5)

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

15. NC1-861
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

1. Symposium: Bronchogenic Carcinoma, Exfoliative Cytology

   Potomac and Virginia Chapters of American College of Chest Physicians, Oct. 31, 1954.

2. Vaginal and Cervical Cytological Studies in Mammary Cancer Patients Receiving Hormone Therapy.

   Transactions of Inter-Society Cytology Council November 1954.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

9. PROJECT DESCRIPTION (Cont.)

Type of Material	Accessions	No. of Slides Examined
Vaginal and cervical	1011	2619
Prostatic	50	270
Gastric	12	67
Bronchial	68	339
Misc. Fluids (includes wound washings)	251	1626
Totals	1392	4921

* These figures do not include the last 10 days of December 1954.

Major findings: At least 3 unsuspected carcinomas of the cervix uteri were detected, two unsuspected lung tumors dedected and the primary diagnosis of two other lung tumors made. Results of wound washings following surgical procedures indicated the presence of tumor cells in several instances.

Studies of carcinoma of the cervix uteri revealed estrogenic argyrophil secretion patterns in all cases but one, although most patients were in the menopause and showed no clinical evidence of estrogen activity.

During endocrine therapy of breast cancer a change in endocrines given could be followed by the study of argyrophil secretion patterns and cytologic details in cells exfoliated from the cervix and vagina.

The presence or absence of estrogenic activity in young girls and in cases of amenorrhea was determined in many clinical cases. The determination of ovulatory and anovulatory cycles was also done.

Significance to Cancer Research: Exfoliative cytology provides an extremely valuable diagnostic tool to the clinician in the vigorous attempt to detect cancer in the earliest possible stage of development so that the possibility of curative therapy may be enhanced.

Exfoliative cytology also provides a method whereby hormone and chemical therapy may be evaluated when it is not possible or practical to remove tumor tissue for biopsy study or in conjunction with biopsy studies.

Exfoliative cytology may, by the study of wound washings after surgery, reveal the answer to the perplexing problem of recurrence of tumors in spite of surgical excision through non-tumor areas.

Studies of cellular components and substances in cells exfoliated by the human or by experimental animals may reveal additional information regarding the differences between malignant and non-malignant tissue.

Proposed course of project: Continuing service and expansion of service to the clinicians of all the categorical Institutes in the Clinical Center, National Institutes of Health. Collaboration and assistance in evaluation of various clinical research projects such as endocrine therapy of cancer, chemotherapy, surgical wound washings and evaluation of hormonal balances in the female. Continued development of new preparation procedures for smears and fluids and new staining procedures, as well as applicability of tissue procedures to smears and fluids.

A continued study of exfoliated cells with special emphasis on cytoplasmic

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